

# Cruise Report



## RV POSEIDON Cruise 525

**Norwegian Sea**

29.06. – 20.07.2018

Compiled by: Janina Büscher

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## 1. Scientific crew &amp; participation

| Name              | Function                | Institute                      | Leg                 |
|-------------------|-------------------------|--------------------------------|---------------------|
| Janina Büscher    | Chief Scientist         | GEOMAR                         | 28.06. – 21.07.2018 |
| Karen Hissmann    | JAGO-Team, coordination | GEOMAR                         | 28.06. – 20.07.2018 |
| Jürgen Schauer    | JAGO-Team, pilot        | GEOMAR                         | 28.06. – 20.07.2018 |
| Peter Striewski   | JAGO-Team, co-pilot     | GEOMAR                         | 28.06. – 20.07.2018 |
| Lina Holthusen    | Student                 | GEOMAR                         | 28.06. – 20.07.2018 |
| Sandra Maier      | Scientist               | NIOZ                           | 28.06. – 20.07.2018 |
| Sandra Brooke     | Scientist               | FSUCML                         | 28.06. – 20.07.2018 |
| Tina Kutti        | Scientist               | IMR                            | 04.07. – 10.07.2018 |
| Narimane Dorey    | Scientist               | IMR                            | 29.06. – 20.07.2018 |
| Øystein Gjelsvik  | Scientist               | IMR                            | 10.07. – 20.07.2018 |
| Nico Schleinkofer | Scientist               | Geosciences, Goethe University | 04.07. – 20.07.2018 |
| Magali Boussion   | Scientist               | CSM                            | 04.07. – 20.07.2018 |
| Daniel Opitz      | Film producer           | Ocean Mind Foundation          | 28.06. – 04.07.2018 |
| Barne Peters      | Camera operator         | Ocean Mind Foundation          | 28.06. – 04.07.2018 |
| <b>Total:</b>     | <b>14</b>               |                                |                     |

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*Cruise legs:*

The cruise legs were subdivided through disembarkation and embarkation of scientific crew

**Leg 1:** 29.6. – 04.07.2018

Bergen – Sula – Nord-Leksa

**Leg 2:** 04.07. – 10.07.2018

Nord-Leksa – Steinavaer – Myre

**Leg3:** 10.07. – 20.07.2018

Hola – Sula – Bergen

**Port Calls:**

04.07.2018 Leksa (Trøndelag County, Norway)

10.07.2018 Myre (Nordland County, Norway)

20.07.2018 Bergen (Norway)

## 2. Research programme

### 2.1 Introduction

(Janina Büscher)

Cold-water corals are important bioengineers of the world's oceans. They provide structural habitat for a diverse species community. However, these fragile organisms, that build their calcareous skeletons from calcium carbonate ( $\text{CaCO}_3$ ), are threatened through human activities like fishing, pollution, and climate change. Ocean acidification, caused by anthropogenic  $\text{CO}_2$  emissions, lead to lowered seawater pH and decreasing carbonate ion concentrations, which in turn results in a diminished saturation state of the oceans with respect to  $\text{CaCO}_3$  (e.g. Caldeira & Wickett, 2003; Feely et al., 2004; Sabine et al., 2004; Orr et al., 2005); consequently affecting the ability of calcifying organisms such as scleractinian corals to build their protective shells and skeletons (e.g. Gattuso et al., 1998; Langdon & Atkinson 2005). At the same time, the corals will experience a steady warming of their environment. Only few studies were carried out to investigate the sensitivity of cold-water corals to several stressors of ocean change (e.g. Hennige et al., 2015; Büscher et al., 2017), and not accounting for interactions of different organisms of the ecosystem. Moreover, *in situ* physiological data on the organism's performance at current status are scarce. Thus, extrapolation of experimental outcomes to the natural environment may be difficult and assumptions on future conditions of cold-water coral ecosystems precarious.

Therefore, the main purposes of the cruise were investigations of cold-water coral ecosystems along the Norwegian coasts and margins from northern-most cold-water coral occurrences to the mid-Norwegian reefs in the Northeast Atlantic with regard to differences in the physiological condition of the organisms of different reef sites. For this, natural growth and degradation processes, the metabolic activity, and other indicators of the physiological performance (i.e. 'fitness', reproduction, lipid composition) of the main framework-forming coral *Lophelia pertusa* and associated key organisms, i.e. the bivalve *Acesta excavata*, were examined by *in situ* as well as on-board experiments and sampling. *In situ* measurements included settlement experiments of marked live and dead coral framework to assess growth and bioerosion rates of *L. pertusa*, as well as incubation experiments to assess community oxygen consumption rates and carbon and nitrogen fluxes. Board incubation experiments were carried out in order to compare *in situ* rates with oxygen consumption and nutrient partitioning under controlled conditions. Moreover, individual *L. pertusa* fragments were incubated under controlled laboratory conditions measuring oxygen consumption of freshly collected specimens under increasing temperatures to identify stress responses of this cosmopolitan species from different reefs. In order to identify genetic variation among and within sites and broaden the picture of the general physiological condition ('health status') of the different reefs/populations, samples for analyses of genetics, reproduction, lipid composition among others were taken. Furthermore, video transect surveys were carried out for species community analysis at all reef sites in order to figure out differences in the community structure of different reefs. Covering the different parameters in different habitats (North vs. mid-Norway and offshore vs. inshore) in presumably different populations of the cold-water coral *L. pertusa* will give us a broader picture of the natural variability of physiological rates and nutrient partitioning of *L. pertusa*.

The physicochemical properties of the water masses surrounding the coral reefs (i.e. temperature, salinity, pH, carbonate chemistry), as well as the food availability and composition (i.e. assessment of organic matter amount of different size fractions) were investigated at all reef sites to compare

biological, physical, and chemical driving forces affecting ecosystem dynamics and functioning. Measurements and sampling were carried out at an offshore reef and an opposing coastal area within Norwegian fjords at both localities, in northern and mid Norway.

The achievements of the cruise – described in detail in the following – will contribute to the understanding of the natural variability in environmental conditions on the rates of biological and physiological processes of *Lophelia pertusa* and associated key species, as well as bioeroders in Norwegian arctic and boreal regions and will, thus, help in future ocean change investigations to define experimental manipulation conditions and interpret results.

## 2.2 Major cruise objectives

(Janina Büscher)

- *In situ* community oxygen consumption and carbon / nutrient flux measurements of *L. pertusa* communities at different reef locations (North and mid-Norway) in their natural habitat to compare the metabolic state among sites and previous laboratory respiration rates.
- Sampling of little fragments of white and orange live *L. pertusa* and perhaps *M. oculata* from spatially distinct colonies of each reef of four different reef sites to determine the general health status/energetic state of different reefs, thereby comparing in- and offshore reefs in North and mid-Norway with regard to reproduction, fatty acid and lipid composition, total proteins, carbohydrates, and metabolites. Moreover, samples from each colony will be taken for genetic analyses to determine the connectivity of the different colonies and reef sites.
- On-board experiments evaluating 1) oxygen consumption of individual *L. pertusa* at ambient seabed temperature as well as increasing temperatures to assess the metabolic differences and stress responses at all four different reef sites, 2) community oxygen consumption of live and dead coral framework in the CUBE systems to be able to compare *in situ* results with more controlled on-board measurements, 3) feeding behaviour of *L. pertusa* from different reefs, 4) the release of dissolved and particulate organic carbon and nitrogen via collection of the mucus of the corals, and 5) the degradation of the mucus over time after collection.
- CTD casts including collection of water samples to characterise the ambient water masses of all reef sites with respect to multiple physical and biogeochemical parameters (temperature, salinity, oxygen, fluorescence, total alkalinity, dissolved inorganic carbon, nutrients, trace elements and isotopes).
- Deployment of *in situ* experiments of live and dead corals in the Sula Reef Complex in order to expand the data set of a former field study from 2013/14 (POS455, POS473) with additional information on natural reef growth and bioerosion after recovery in 2-3 years.
- Sampling of living and dead specimens of *Lophelia pertusa* and associated organisms with the manned submersible JAGO for advanced laboratory experiments (i.e. spectral imaging and photogrammetry of live coral fragments within their medium in aquaria to document expansion of polyps and determine the size of the fragments in terms of volume / surface area in complex 3-dimensional image analysis).

### 3. Narrative of cruise with technical details

(Janina Büscher)

**Note:** In the following cruise narrative, station numbers with Roman numerals (I - IV) refer to the main study areas (see Appendix: A. Maps of cruise stations), whereas Latin numerals (1-63) refer to the internal continuous station numbers of RV POSEIDON cruise 525 (see Appendix: B. Station list). All times are given in local time.

#### 28<sup>th</sup> June 2018

Loading of scientific equipment and embarkation of most scientific cruise participants (see table in chapter 1 for details).

#### 29<sup>th</sup> June – 1<sup>st</sup> July 2018

At 9:00 a.m. RV POSEIDON left the 'Dokkeskjaerskajen' in Bergen and headed towards Station I, the Sula Reef Complex (Fig. 2, and Appendix figures A1+A2). During transit, facilities and equipment for scientific on-board experiments and cultivation of *Lophelia pertusa* and associated fauna were set up.

#### 1<sup>st</sup> July 2018

At station in Sula (I), we started immediately with one of our main tasks for this cruise, the deployment of *in situ* incubation chambers (CUBEs) to investigate community oxygen consumption rates as well as nitrogen and carbon fluxes of an intact coral fragment of live and dead *Lophelia pertusa* and associated organisms (see chapter 4.2). Before CUBE deployment, the operation with research submersible JAGO was practiced for the ship's crew to get a feeling for deployment and recovery of the submersible (St.1-1). Afterwards, two CUBEs were deployed one after another by means of hanging them below the CTD frame (Fig. 1) and releasing them above the seafloor with an acoustic releaser unit attached to the frame of the CTD (St.2-1, 3-1). This could be done without exploration dive with JAGO before, since we know the area quite well and were certain to deploy the CUBEs onto sediment underground. With JAGO we searched for the two deployed CUBEs (Dive #1; St.4-1), set them up in the right position and placed a coral fragment, which we collected before arrangement of the CUBEs from the closest reef structures, underneath one CUBE. The other CUBE was placed over bare sediment. The CUBEs were then started by moving a handle by means of JAGO. The stirrers inside started rotating, which was a good indication that the programme had started.



**Fig. 1:** Deployment of a CUBE with the CTD / water sampling instrument. (Picture: Janina Büscher)

## 2<sup>nd</sup> July 2018

The second working day started with a JAGO dive (#2; St. 5-1) and was conducted as survey to document the reef community via video records and GoPro still imaging. In the afternoon, deep sea water was pumped from 100 m water depth by means of a water pump attached to the CTD frame (St. 6-1; see also chapter 5.3). The pumped deep water was filled into the set up cultivation tanks and experiment facilities and into the ship's own aquaria tanks as reservoir.

## 3<sup>rd</sup> July 2018

The first JAGO dive of this day (#3; St. 7-1) was conducted to recover the CUBE systems after 24 hours of measurement, which was postponed from yesterday afternoon due to high swell. Both CUBEs were successfully retrieved from the seafloor with JAGO and brought back on board with the help of the life boat. Afterwards, a CTD (St. 7-1) was carried out to characterise the water column and to take water samples. The last dive in Sula (#4; St. 9-1) was accomplished as a sampling dive in order to collect corals for *in situ* as well as on-board incubation experiments and smaller fragments to be preserved for later analyses such as population genetic, reproduction, and lipids in the laboratories of different collaborators. Afterwards, water from 100 m depth was pumped again to fill the ship's aquaria tanks (St. 10-1). Around midnight we headed towards the second station 'Nord-Leksa' (Station II) in the outer Trondheimsfjord.



**Fig. 2:** Reef locations along the coast of Norway at different latitudes and environments with two inshore (II and IV) and two offshore (I and III) reef sites.

## 4<sup>th</sup> July 2018

In Nord-Leksa we conducted a first dive in the morning (#5; St. 11-1/#6; 12-1) for exploration purposes and video surveys. Due to an error in the USBL connection, we had to cancel the first trial and re-launch JAGO later that morning. Afterwards, a crew exchange took place via tender. The camera team consisting of two persons was dropped off and three scientists, including Tina Kutti, who had to postpone embarkation due to illness, were picked up. In the afternoon, a CTD cast (St. 13-1) was conducted, including water sampling, to characterise the water column.

## 5<sup>th</sup> July 2018

Before the first JAGO dive, a CTD cast (St. 14-1) was carried out to characterise the water column (including water sampling). At 8:30 a.m. JAGO went down with the purpose of a transect dive to document the reef community and status in Nord-Leksa (#7; St. 15-1). Afterwards, CTD casts were conducted to characterise the water masses on the reef top as well as on the reef flanks with no reef elevation (St. 16-1 – 19.1).

**6<sup>th</sup> July 2018**

Like yesterday, we started with a CTD cast (St. 20-1) before going down with JAGO for a collection dive in Nord-Leksa (dive #8; St. 21-1). A final CTD cast at this station was carried out after lunch to finalise the water sampling (St. 22-1), after which we started heading towards the northern reef sites Hola and Steinavaer (Fig. 2, Appendix A1, A4+A5).

**7<sup>th</sup> July 2018**

Transit to the northern cold-water coral reef locations, passing the Lofoten in the evening. At 9:30 p.m. we intermediately stationed in the harbour of Myre, our port of call for the 10<sup>th</sup> of July, in order to collect broken spare parts of the life boat by means of the dinghy from a workshop onshore. Due to relatively turbulent weather conditions in the offshore area, we headed straight to the inshore reef site 'Steinavaer' (Station IV).

**8<sup>th</sup> July 2018**

We started with our routine work in our third approached research area, Steinavaer (Station IV), at 8 a.m. in the morning with a CTD cast (St. 23-1) followed by a JAGO dive (#9; St. 24-1). The first dive was carried out for exploration of the for us relatively unknown reef area. Two more CTD casts (St. 25-1, 26-1) on the flanks of the reef structures were carried out after the JAGO dive. A second JAGO dive (#10; St. 27-1) was carried out in the afternoon to sample *L. pertusa* fragments from this site for on-board experiments and preservation for different analyses (see above).

**9<sup>th</sup> July 2018**

This day, two exploration and coral sampling dives were carried out (#11; St. 29-1 and #12; St.32-1), embraced by CTD casts (St. 28-1 in the morning, St. 30-1 and 31-1 at lunchtime, and St. 33-1 in the evening) to characterise the water column on the reef top, as well as on the flanks, including water sampling for seawater chemistry analyses and organic matter abundance.

**10<sup>th</sup> July 2018**

We docked at Myre at 9 a.m. to conduct buoyant weighing of cold-water coral fragments for *in situ* deployment experiments to be deployed at Sula Reef and for another crew exchange. Disembarkation of Tina Kutti took place at 10.30 a.m. and Øystein entered the vessel at ~ 3 p.m. In the evening, we cast off in direction of Hola (Station III).

**11<sup>th</sup> July 2018**

At Hola, we started our routine work with a CTD cast (St. 34-1) above the reefs. Due to unsuitable weather conditions for JAGO, we launched the CTD again to approximately 100 m water depth with the water pump attached to fill up the water reservoirs and ship tanks with fresh deep water (St. 35-1). After lunch a first JAGO dive at Hola could be carried out (#13; St. 36-1) for exploration of the habitat for suitable CUBE deployment positions.



**12<sup>th</sup> July 2018**

Due to unsuitable weather conditions for JAGO, CTD casts were carried out before noon only (St. 37-1 – 39-1) to characterise the water masses above the reef and on the flanks of the Hola reefs, including water sampling. In the afternoon, the CUBE systems were deployed once more one after the other with the CTD (St. 40-1, 41-1). The following JAGO dive was conducted with the purpose of positioning the CUBEs, and to equip one CUBE with a coral and start the 24-hour measurement (#14; St. 42-1). Unfortunately, the CUBEs were not found before JAGO had to lift off and come back on deck.

**13<sup>th</sup> July 2018**

Therefore, after the routine morning CTD (St. 43-1), the first JAGO dive (#15; St. 44-1) of today was dedicated to the search of the CUBEs. After finally finding them again, the measurement was started according to earlier procedures. After lunch, two CTD casts were carried out to characterise the thermocline in more detail at different stations (St. 45-1, 46-1). In the afternoon another JAGO dive (#16; St. 47-1) was performed to sample coral colonies for the on-board incubation experiments.

**14<sup>th</sup> July 2018**

The CTD casts for water mass characterisation and water sampling was finalised this day with one usual CTD in the morning (St. 48-1), after which the first JAGO dive (#17; St. 49-1) was conducted as a transect dive for reef health and community analysis. After lunch, three casts were carried out (St. 50-1 – 52-1). The afternoon JAGO dive (#18; St. 53-1) aimed at retrieving the CUBE systems, as well as the incubated coral. After successfully finalising all tasks at Hola with this dive, we started heading back towards the southern research areas.

**15<sup>th</sup> July 2018**

Transit to the Sula Reef Complex (Station I).

**16<sup>th</sup> July 2018**

In Sula, we started with a JAGO dive (#19; St. 53-2) straight away when arriving at station to collect coral samples and document the reef site chosen for *in situ* as well as CUBE experiment deployment. After a CTD cast (St. 54-1) to characterise the water column, the following two CTD casts (St. 55-1, 56-1) were carried out to release the two CUBE systems onto the seafloor. The following JAGO dive (#20; St. 56-2) aimed at finding and positioning the CUBEs and equip both of them with suitable coral fragments to have a replication of three corals at Sula.

**17<sup>th</sup> July 2018**

This day comprised a perfect “routine day” with a CTD cast (St. 57-1) in the morning, followed by a JAGO dive (#21; St. 58-1) for documentation purposes. After lunch, three CTD casts were conducted to complement the water mass characterisation on top of the reefs as well as the flanks at Sula (St. 59-1 – 61-1). Another documentation dive with JAGO (#22; St. 62-1) took place in the afternoon. In the evening, the CTD with the water pump attached was launched to 100 m to fill up all systems and the ship’s aquaria tanks with Sula deep water (St. 62-2) until midnight.

**18<sup>th</sup> July 2018**

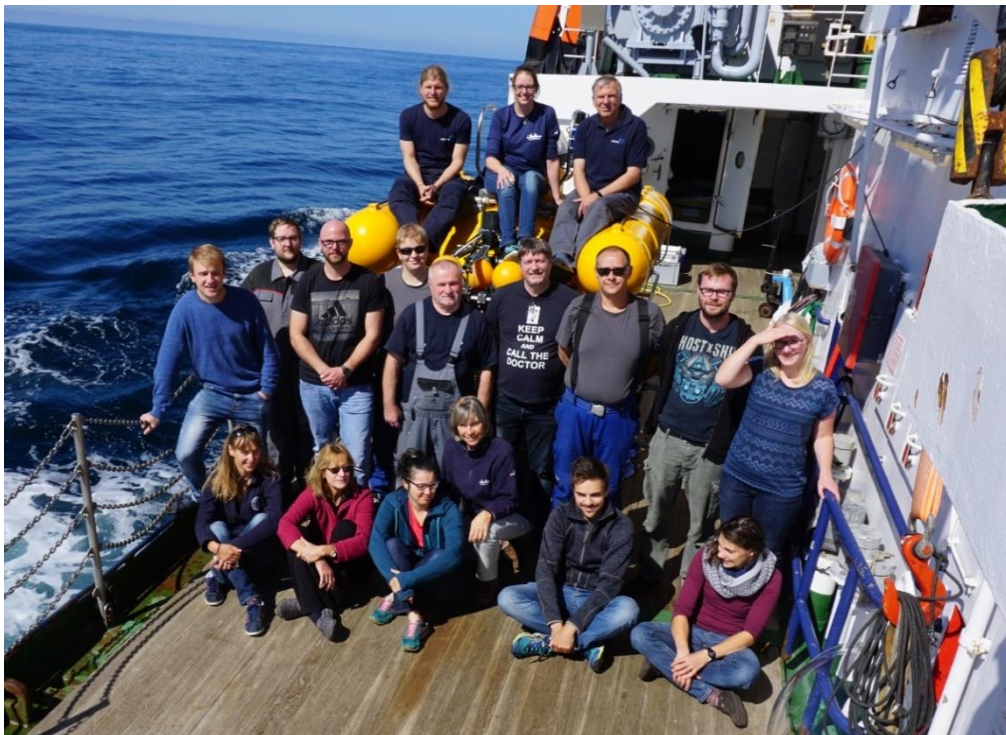
The last JAGO dive of this cruise (#23; St. 63-1) was conducted on this day to bring down the *in situ* experiments of marked and weighed live and dead corals and to recover the CUBEs. The dive started at 9:30 a.m. after preparations with 4 coral baskets and a dead coral framework cluster in the sampling box of JAGO, as well as the hooks for CUBE recovery and sediment sampling equipment. On the surface, two of the coral baskets, as well as one sediment sampling cylinder were lost due to wave action while pulling JAGO with the life boat. JAGO landed directly next to the CUBEs on the seabed. After deployment of the remaining *in situ* experiment baskets in the nearest reef structures, we started searching for the lost baskets, which we indeed found after some trials in the end! We collected them back into the sampling basket of JAGO, went back to the deployment position and placed them next to the first ones. Coming back to the CUBEs, we first checked the coral status, tightness as well as functionality of the CUBEs, pushed them over to reach the coral fragments and sampled the corals before hooking up the CUBEs and descending from the bottom. As soon as JAGO was safely back on board, we started our transit back to Bergen at 4 p.m. in the afternoon.

**19<sup>th</sup> July 2018**

During transit to Bergen, last on-board measurements continued and scientific equipment not needed anymore was packed.

**20<sup>th</sup> July 2018**

We moored at the 'Festningskaian Nr. 1' in Bergen at 9 a.m. Discharge of scientific cargo after administrative clearance (immigration, customs etc.) and disembarkation of scientific participants went smoothly. End of the scientific cruise POS525.



**Fig. 3:** Group picture of the POS525 crew (some ship crew members missing).

## 4. Measurements & sampling, and preliminary results

### 4.1 *In situ* growth and bioerosion experiments

(Janina Büscher)

#### Goals

The *in situ* growth experiments conducted in 2013 and 2014 during the cruises POS455 and POS473 (see cruise reports by Form et al., 2014; 2015) shall be repeated in Sula, as in the years before the deployment of the coral baskets were only carried out on soft sediment underground in Sula and about 50 – 100 m away from live coral reef structures. However, the results of the first experiment deployed in Sula and Nord-Leksa (on- and off-reef) showed a much higher variability in growth rates of corals directly near the corals in Nord-Leksa, while lower growth rates were gathered in Sula where corals were deployed on soft sediment only (Büscher et al., to be submitted soon). Since the data from the mentioned experiment are therefore difficult to compare and interpret and a distinct difference could not be detected or attributed to the location, the *in situ* growth experiment with live and dead coral framework shall be repeated at the Sula reef site, but this time directly in the reef structures as in Nord-Leksa in 2013-2014. Moreover, two different staining methods shall be compared in this new approach and the coral cages are supposed to stay longer in the habitat, especially for the bioerosion processes to be more distinctive than after only a relatively short time period for bioerosion of one year (Büscher et al., to be submitted).

#### Measurements and methods

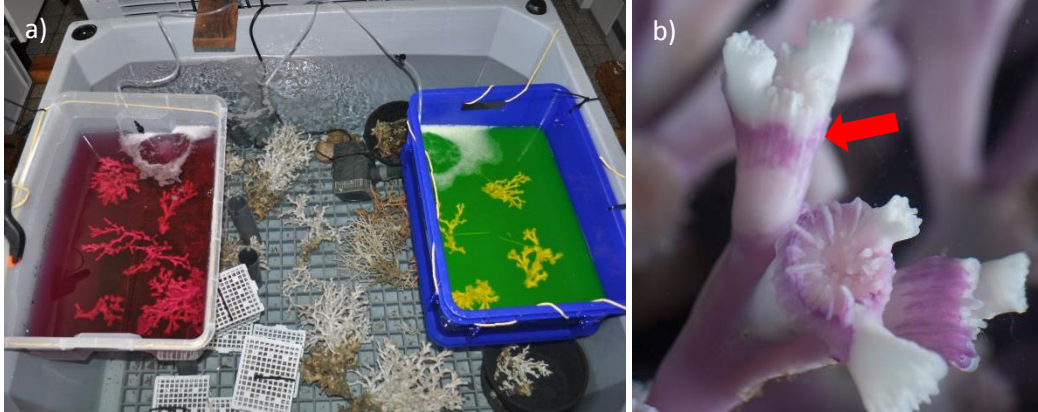
PE autoclave baskets equipped with stained and weighed white and red *L. pertusa* fragments as well as a cluster of six smaller baskets with weighed dead coral framework were successfully deployed at a reef mound at the edge of the Sula Reef Complex into reef structures with live and dead coral cover similarly to the deployments in the Nord-Leksa reef area during POS455 in 2013 (Fig. 6). The weighing prior deployment took place onshore in Myre, since weighing on board is rather difficult due to the ships' movements and vibration. For this, a large water tank of ca. 60 L volume with the selected coral fragments for this experiment was carefully transferred to the shore by means of the ship's crane, where it was connected to a cooling unit and pumps to sustain cultivation conditions of the corals. Next to this water tank was a separate smaller aquarium installed, into which the coral branches were transferred one after another, and weighed using the buoyant weighing technique (Davies, 1989). Above this water bath a high precision analytical balance (Sartorius CPA225D, readability: 0.1mg) with an underwater weighing facility was mounted (Fig. 4). The corals were thus hung into a hook on a thin wire underneath the balance with a Nylon thread attached to each coral branch. After weighing of every coral, the water in the weighing aquarium was replaced by fresh new cold water from the bigger cultivation tank,



**Fig. 4:** Set-up of weighing facility on-shore in the port in Myre in front of the RV POSEIDON in order to weigh coral branches under water in a calm environment. Picture: Janina Büscher.



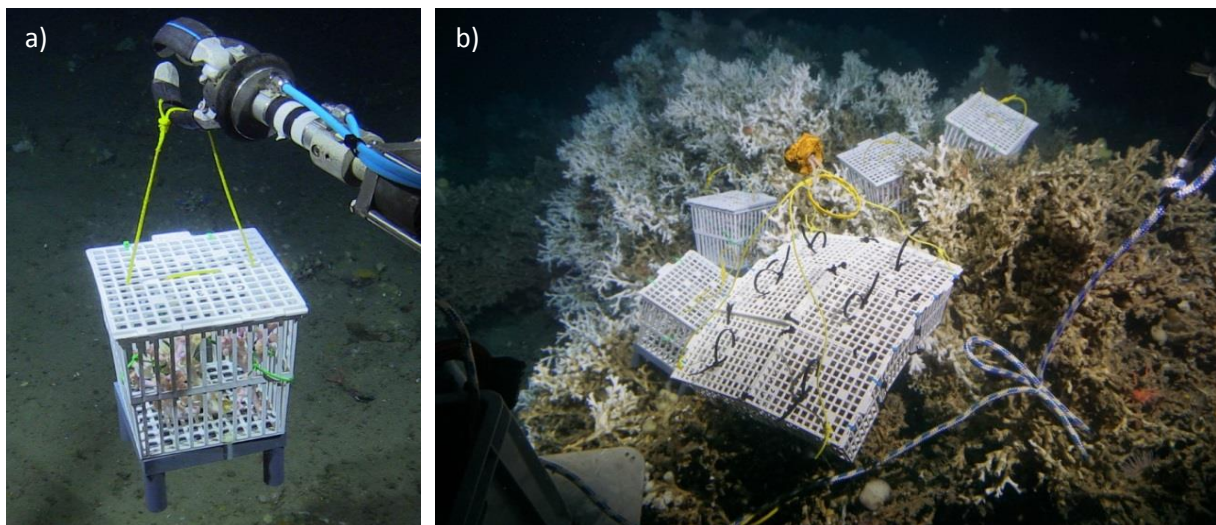
before the next coral branch was inserted. After recovery of the corals from this procedure, the weighed coral branches were stained for a couple of days in Alizarin Red S or Calcein enriched water in separate containers on board (Fig. 5a). These dyes are incorporated into the skeleton of the polyps during calcification and show a growth band at the time of staining from which new growth can be measured linearly in the aftermath (Fig. 5b).



**Fig. 5:** Staining of coral fragments on board RV POSEIDON in separate staining tanks cooled by using large cultivation tanks as water baths (a), and (b) dye marks of a stained coral branch a year after staining. Pictures: a) Karen Hissmann, b) Janina Büscher.

### Preliminary results

During launching of JAGO on the last day of research of the cruise (18<sup>th</sup> July), two of the coral baskets got lost due to wave action when JAGO was pulled at the sea surface with the life boat, as explained in the narrative. Fortunately, the missing baskets could be retrieved and positioned next to the others into the reef in Sula (Fig. 6), where they will grow and in case of the dead corals erode for 2 to 3 years from now on until they will be recovered and analysed with respect to weight gain or loss, as well as polyp length growth in terms of the living coral branches. Moreover, tissue and skeleton overgrowth of white and orange specimens will be examined as observed between both colourmorphs during POSEIDON cruise POS420 in 2011 in Norwegian reefs (see Hennige et al., 2014 and Form et al., 2011\_Cruise Report POS420).



**Fig. 6:** *In situ* coral baskets with live labelled corals and a cluster of bioerosion baskets with dead coral fragments lifted with JAGO's manipulator arm (a) and deployed into the reef framework near living *Lophelia* colonies (b). Pictures: a) Karen Hissmann, b) Janina Büscher.

## 4.2 Cold-water coral community biogeochemical cycling *in-* and *ex-situ*

(Sandra Maier)

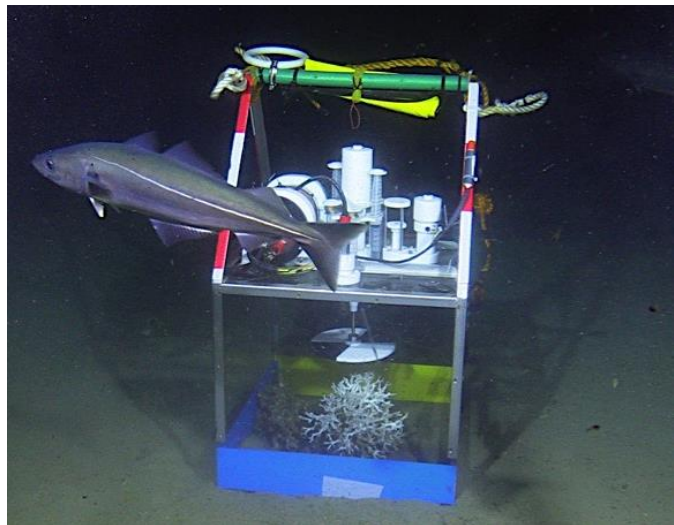
### Goals

The aim of the present study was to assess the biogeochemical cycling of an entire cold-water coral (CWC) reef community, including live *L. pertusa* as well as the coral framework-associated community, both *in-* and *ex-situ*, of coral colonies from different reefs and sites. Hereby, the partitioning of oxygen consumption amongst the community members as well as carbon and nutrient fluxes played a major role in all assessments. *In situ* measurements may give more reliable quantifications of biogeochemical cycling than *ex situ* incubations, which may be influenced from stress-induced changes in physiological rates due to handling and artificial maintenance (Khripounoff et al., 2014). Nevertheless, *ex situ* incubations provide an important tool to experimentally assess the physiological consequences of global climate change (Turley et al., 2007). This study provides a direct comparison of *in situ* and *ex situ* measurements with incubation chambers (CUBEs) described in the following.

### Measurements and methods

#### ***In situ* CUBE incubations**

To assess the biogeochemical cycling of the CWC reef community *in situ*, newly developed large (0.5 x 0.5 x 0.5 m<sup>3</sup>) automated *in situ* incubation chambers (CUBEs, Stratmann et al., 2018) were deployed close to the coral reefs, i.e. starting with the first approached location – the Sula Reef Complex (Fig. 7). For each deployment, two CUBEs were lowered subsequently to the near-reef soft-sediment seabed underneath the CTD frame and released by acoustic release 5–10 m above the seabed. In a subsequent dive with submersible JAGO (see also section 5.1), one to two ca. 25 cm x 25 cm



**Fig. 7:** *In situ* CUBE incubation of a coral fragment in Sula.

Picture: JAGO-Team.

x 25 cm blocks of dead coral framework including some live coral generations still attached ('framework-coral blocks') were collected with the manipulator arm of JAGO (section 5.1) and carefully translocated to the soft-sediment area, where the CUBEs were located. The CUBEs were then placed over the 'framework-coral blocks' with the manipulator arm and sealed by slightly pressing them into the sediment. Seal tightness was examined visually by circuiting the CUBEs with JAGO. Extant gaps between the CUBE edges and the sediment surface were sealed by piling up more sediment at the CUBE edges. In addition to the incubated framework-coral blocks, an 'only-sediment' incubation served as control to correct oxygen and nutrient cycling rates for the respective rates of the sediment without coral and associated fauna. The incubation was enabled by

activating the start-signalling handle of the CUBEs by means of JAGO, which started an internal programme saved prior to the deployment to run autonomously for 24 hours on the seafloor. A stirring plate kept the water in the CUBE in motion, providing flow and preventing concentration gradients within the CUBE. A deep-sea rated optode (Contros Hydroflash<sup>®</sup> O<sub>2</sub>; Kongsberg Maritime Contros GmbH, Germany) continuously measured the oxygen (O<sub>2</sub>) concentration at a frequency of one or 30 measurements per second. After an acclimatisation time of 10 minutes an inert bromide tracer was injected via an O-ring-sealed injection port on top of the CUBEs. The CUBE water sampling rosette with six 35 mL syringes attached to the O-ring sealed water sampling ports on top of each CUBE allowed water sampling to measure concentrations of the bromide tracer to check for leakage, as well as dissolved nutrient concentrations. The first water sample was taken 10 minutes after the bromide addition, the following five at time steps of every 4.8 hours. After an incubation time of 24.5 hours, the measurements stopped automatically and the CUBEs were recovered with JAGO thereafter. The coral-framework blocks were collected by means of JAGO into the attached sampling basket, and a sediment sample was scooped into a sediment sampling cylinder.

### On-board CUBE incubations

On-board CUBE incubations (Fig. 8) were carried out on deck of RV Poseidon in a large tank (ca. 3000 L) with circulating 'in situ' water collected from 50 m water depth at the Sula reef station (see section 5.3). The water was permanently cooled to a temperature of 7.2 – 7.7 °C and the tank was covered with thick black plastic bags to provide darkness and minimize the disturbance of the organisms during the incubations. Framework-coral blocks were collected from Nord-Leksa (Station II, Fig. 2), Steinavær (Station III), and Hola reefs (Station IV) and maintained in large holding tanks (see section 5.4). The CUBEs were



**Fig. 8:** On-board CUBE incubation of a coral fragment. Picture: Sandra Maier.

sealed at the bottom with thick PE-foil, attached to the CUBEs with PE-tape, and immersed in the water tank with the side door open to fill them with water. Framework-coral blocks were carefully placed inside the CUBEs through the side door. During handling, great care was taken not to expose the blocks to air. Framework-coral blocks were allowed to acclimatise for at least 30 minutes inside the CUBEs, during which the side door remained open and a stream pump ensured water movement within the CUBEs. The incubation was then carried out with the same programme as described for the *in situ* incubations. 'Only-water' control incubations were carried out for the *ex situ* incubations, too, to correct rates of oxygen consumption and nutrient cycling of the framework-coral community for the microbial activity in the surrounding water. Two on-board CUBE incubations could be carried out at the same time in the large water tank similar to *in situ* incubations.

## Individual incubations

Individual incubations of (a) macrofauna, (b) live corals and (c) 'dead' coral framework ('framework') including associated endofauna, microfauna and microorganisms were carried out after the on-board CUBE incubations, to derive their individual rate of the total community oxygen consumption. Macrofauna and live corals were carefully separated from the 'framework', while remaining submersed to reduce their disturbance as much as possible. Macrofauna was sorted to the lowest resolution possible (i.e. brittle stars, bivalves, brachiopods, etc.) and incubated separately in 60 mL falcon tubes submersed in the cooled, darkened water tank. Corals and 'framework' were separately incubated in 800 mL incubation chambers (see section 4.3). A two-point measurement of O<sub>2</sub> concentration with the Contros optodes were carried out to determine oxygen consumption rates at the beginning and end of the individual incubations.

## Sampling of framework-coral blocks

After the respective incubation, the volume of each incubated framework-coral block and individually incubated member of the reef community was assessed via its water displacement. Macrofauna was stored frozen for laboratory analysis of dry-weight specific carbon and nitrogen content. Subsamples of the live coral and 'framework' were stored frozen, while the remainder was separately oven-dried at 40 °C for dry weight assessment.

## Preliminary results

A total of four *in situ* CUBE incubations were carried out at Sula, including three incubated framework-coral blocks and one 'only-sediment' control incubation (Table 1). The *in situ* deployment of the CUBEs was restricted to the offshore reefs, which were the only reef sites with relatively fine, soft sediment in close vicinity. The inshore reefs were surrounded by much coarser sediment, with many stones and coral rubble, which obstructed a successful sealing of the CUBEs. A future protocol for *in situ* CWC reef community incubations can be developed based on this primary study. Unfortunately, the coral measurement in the Hola reef area can not be analysed, because the CUBE overturned with unknown point in time to assess a valuable measurement.

On board, five framework-coral blocks from three different reefs (Table 1) were incubated in the bottom-sealed CUBEs, accomplished by three only-water control incubations. From four on-board CUBE incubations, subsequent sets of individual incubations were carried out.

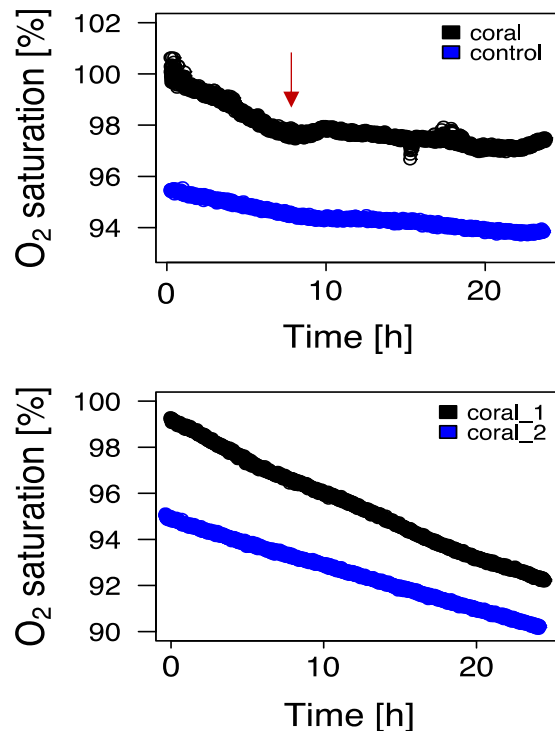
**Table 1:** Overview on implemented incubations.

| Site       | CUBE incubations |         |          |         | Individual<br>fauna<br>incubations |
|------------|------------------|---------|----------|---------|------------------------------------|
|            | <i>In situ</i>   |         | On board |         |                                    |
|            | coral            | control | coral    | control |                                    |
| Sula       | 3                | 1       | 0        | 0       | 0                                  |
| Hola       | (1)              | (1)     | 2        | 1       | 2                                  |
| Nord-Leksa | 0                | 0       | 1        | 1       | 0                                  |
| Steinavær  | 0                | 0       | 2        | 1       | 2                                  |



A decrease in oxygen saturation was observed in all *in situ* CUBE deployments (Fig. 9). At the recovery of one *in situ* CUBE deployment, a gap was observed between a CUBE corner and the soft sediment. The resulting leak and water exchange can be tracked in the respective oxygen saturation profile (Fig. 9, arrow in top graph).

Oxygen concentrations and consumption rates will be calculated from the obtained raw data, and normalised to the carbon content of the incubated framework-coral community. Water samples were retrieved from two *in situ* CUBE incubations (not during the first two deployments due to technical issues with the syringes coming up), and from all on-board CUBE incubations, and will be analysed for nutrient and tracer bromide concentrations.



**Fig. 9:** Decrease of O<sub>2</sub> saturation during the *in situ* incubations as a result of CWC community respiration. The arrow indicates the start of a leak in one of the CUBEs.

#### 4.3 On-board temperature stress experiments of different *L. pertusa* populations

(Øystein Gjelsvik, Narimane Dorey, Tina Kutti)

##### Goals

Temperature in the ocean has been rising for the past decades and is expected to increase in the years to come (IPCC, 2014). Such a change has multiple effects on the physical conditions in the ocean and will represent multiple stressors for deep-sea organisms used to a relatively stable environment.

During the POS525 cruise, we ran an on-board experiment to investigate the effect of thermal stress on the abundant cold-water coral species *Lophelia pertusa*. The coral has a great impact on ecosystem processes in its vicinity, working as a base for attachment for other organisms (Freiwald et al., 2004), hosting a large bacterial diversity (Jensen et al., 2008) and with the reef itself known to harbour more than 1800 species in the NE Atlantic and 2700 species known worldwide (Roberts & Cairns, 2014). We expected different populations of *L. pertusa* in Norwegian waters to have different physiological responses to increasing temperature. In particular, we hypothesized that populations closer to the shelf (e.g. inside fjords) experience more temperature variations than populations further away and will therefore be more resistant to an increase in temperature. Although little is known on cold-water corals, Carilli et al. (2012) demonstrated that tropical corals exposed to larger temperature fluctuations through the year were more resistant to short warm-water events. Hence,



the past and current temperature variability might play a key role in determining the resilience to temperature stress in tropical shallow water corals, and perhaps likewise in cold-water corals.

Here, we investigated the response of shelf and fjord *L. pertusa* populations to temperature stress by measuring respiration rates, mortality, behaviour, and cellular stress markers. This study aims at improving knowledge on the sensitivity of different populations of the ecologically important *L. pertusa* to future oceanic changes.

### Measurements and methods

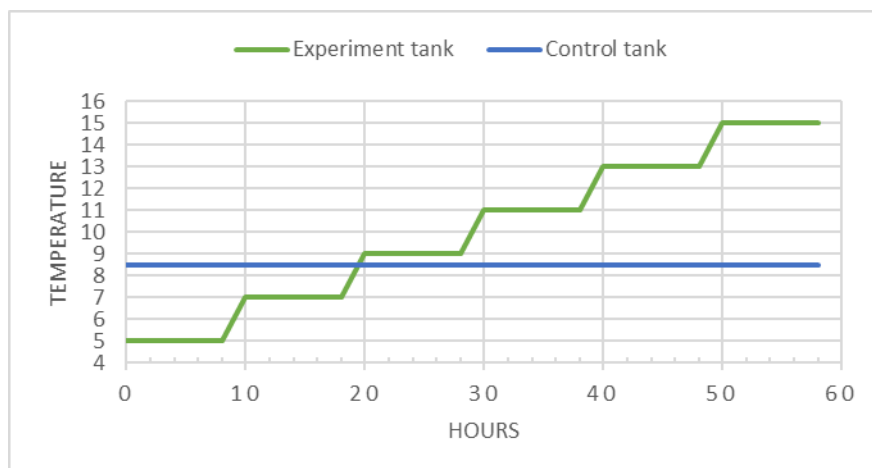
#### **Coral collection**

Specimens for the on-board experiment were collected at all four reefs along the Norwegian coast (Fig. 2). At each reef, five to seven pieces of live corals were collected using the submersible JAGO (see chapter 5.1) from different locations within the reefs, and each sample was assumed to be from a different colony.

After being brought up to the surface, corals were immediately transferred to two connected PVC tanks of 3000 litres in total filled with re-circulating seawater cooled to seabed temperature (see chapter 5.4) without being subjected to air. Specimens were acclimatised to the tank conditions for several hours before being transferred to the experimental tanks.

#### **Experimental set-up**

For each reef, we started the experiment by fragmenting corals from five different colonies into pieces of approximately 10 – 15 cm length for respiration and few smaller pieces for stress marker analyses. The pieces were transferred to a tray floating inside two square tanks of 1000 litres, each connected to a cooler (Aqua Medic Titan 1500, 790 Watt). One of the tanks, the “control tank”, was kept at 8.5 °C for the entire experiment duration. The other tank, the “experimental tank”, was the temperature treatment tank, which was increased in temperature over the course of the experiment by means of a heater (300 W) and a regulator (InkBird ITC-308) in addition to its cooler. At the start of the experiment, the experimental tank was set to 5 °C and temperature in the tank was increased from 5 to 15 °C by steps of 2 °C every 8 hours: corals were exposed to 5 °C degrees for 8 hours, before the temperature was increased gradually by two degrees for two hours at each step (Fig. 10). Each experiment, one for each of the four reefs, lasted 58 hours in total.

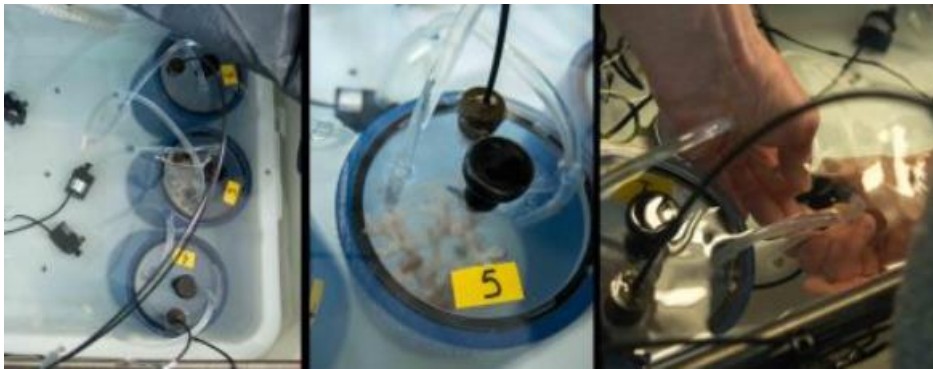


**Fig. 10:** Illustration of temperature manipulation in the experimental and control tanks.

Seawater was exchanged before the start of each experiment, with new unfiltered seawater being pumped up from 100 meter water depth at the offshore locations Sula and Hola (see section 5.3). Temperature was measured continuously in the experimental tank using a LabQuest2 (Vernier) with the associated Surface Temperature Sensor. Temperature and salinity were punctually measured in both tanks using a conductivity meter with an integrated thermometer (Cond 3210, WTW GmbH, Weilheim, Germany).

### Respiration measurements

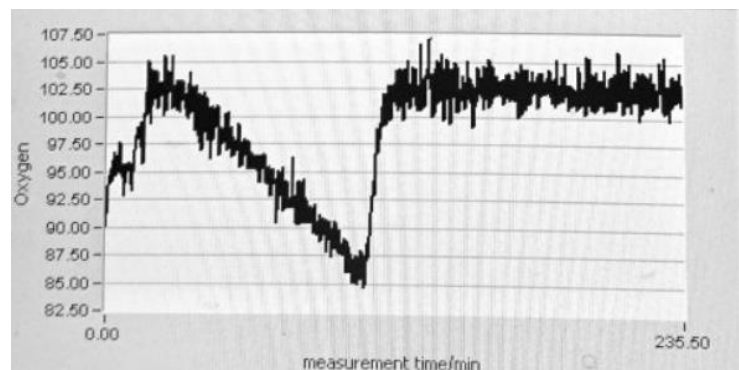
At the start of the experiment, we enclosed each of the five coral fragments in a respiration chamber (Fig. 11). The experiment tank contained six chambers for respiration measurements: one for each coral fragment and an empty one without any fragment used as a control for bacterial background respiration analysis. In addition to this, the control tank contained two respiration chambers: one with a colony corresponding to one of the five colonies used in the experimental tank and one empty control. The control tank aimed to help monitoring a possible drift in coral oxygen consumption over time during the experimental period of each measurement round.



**Fig. 11:** Respiration chambers with *L. pertusa* inside and connected to a circulation pump and an oxygen sensor. Picture: Narimane Dorey.

Respiration was measured in 8 chambers in total for 1 to 8 hours. During the measurements, the seawater flow was kept constant inside the chamber ( $0.54\text{--}0.69\text{ L min}^{-1}$ ) by small water circulation pumps (SAILFLO, 5V). Oxygen never dropped below 70 % oxygen saturation. During the time when respiration was not measured, the water circulating pumps were disconnected from the chambers at the inflow to re-circulate new water from the surrounding tank into the chambers and oxygen quickly rose up back to 100 % (Fig. 12).

Oxygen measurements were conducted with optical probes (PreSens Polymer Optical Fibre with planar oxygen-sensitive spots) mounted on a 10-channel fibre optic oxygen meter (PreSens Oxy-10 Mini). Oxygen concentration and atmospheric



**Fig. 12:** Screenshot of a running respiration measurement logging of one coral fragment, illustrating a gradual decrease of oxygen before opening the chamber. Picture: Øystein Gjelsvik.

pressure were noted at the end of each respiration run, and water was sampled for later measurement of ammonia. Corals were then sampled into vials and immediately frozen at -80 °C for further analyses of the dry / ash weight for normalisation of the respiration data.

### **Stress marker analyses**

Duplicates of small fragments from the five colonies were kept labelled in a tray with flowing water inside both tanks (n = 5x2 fragments in each tank). One of the duplicates was frozen with liquid nitrogen and put in a -80 °C freezer for later stress marker analysis in the lab, and the other one was prepared for lysosomal membrane stability (LMS) analysis on board.

#### *Lysosomal membrane stability:*

Lysosomal membrane stability was measured using a neutral red retention assay (bivalves: Edge et al., 2015; corals and sponges: Edge et al., 2016). Chemicals were mixed according to the protocol (Edge et al., 2016), and coral pieces prepared on board by homogenising tissue in a mortar and adding chemicals as described in the protocol. Cells were incubated at room temperature in the dark with a neutral red dye. The retention of neutral red in lysosomes was examined using light microscopy. This allows for the investigation of the ratio of intact vs. destabilised stained *L. pertusa* cells.

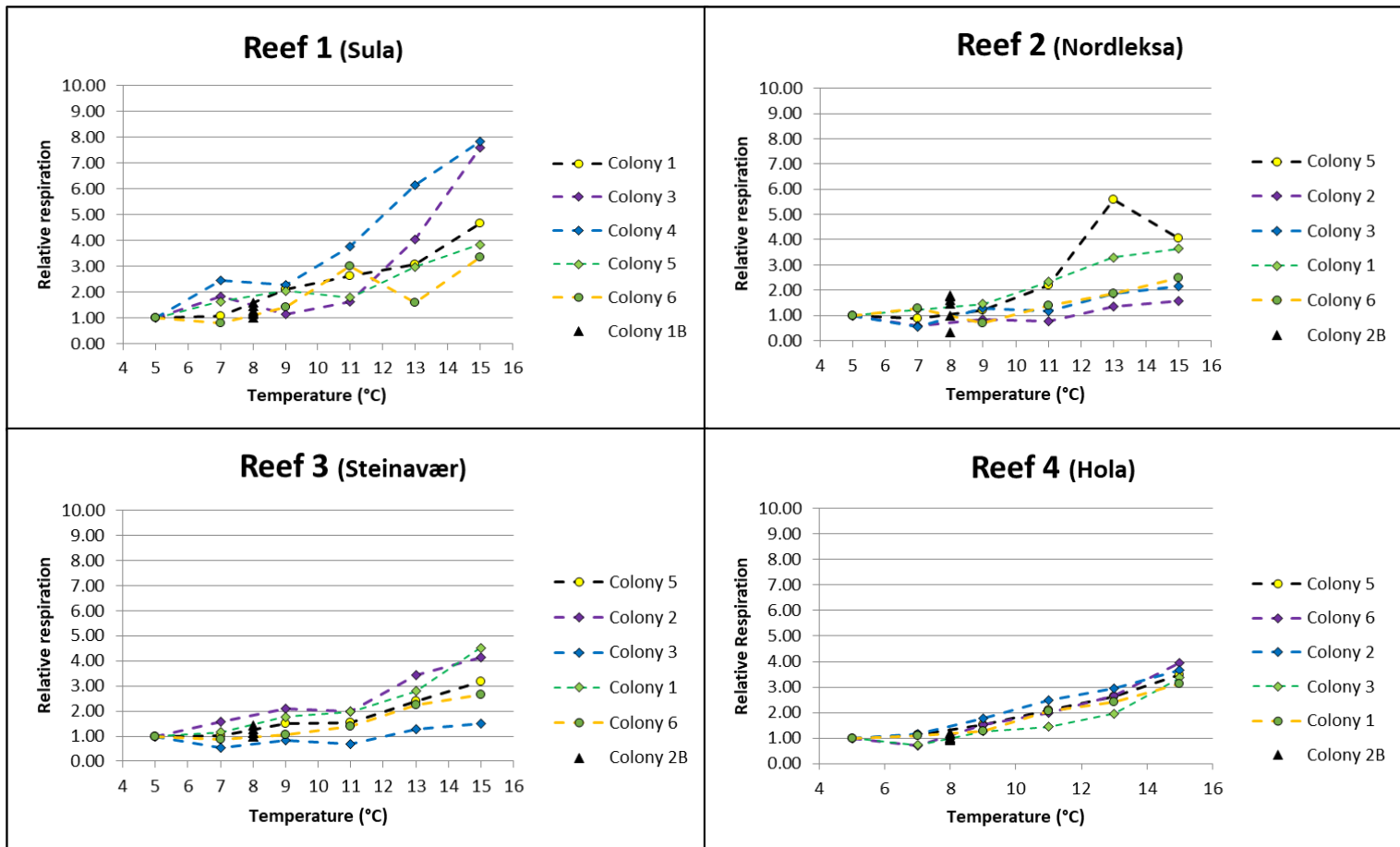
#### *Lipid peroxidation:*

In order to identify oxidative damage to cell membranes, lipid peroxidation will be analysed as a stress marker using a modified protocol of Ringwood et al. (2003). Frozen tissue will be homogenised, and a dye detecting malondialdehyde (end-product of oxidatively damaged membrane lipids) will be added. Samples will then be analysed with a spectrophotometric assay. This is expected to be completed by end of 2018 and will give better indications of the general stress levels in the corals.

### Preliminary results

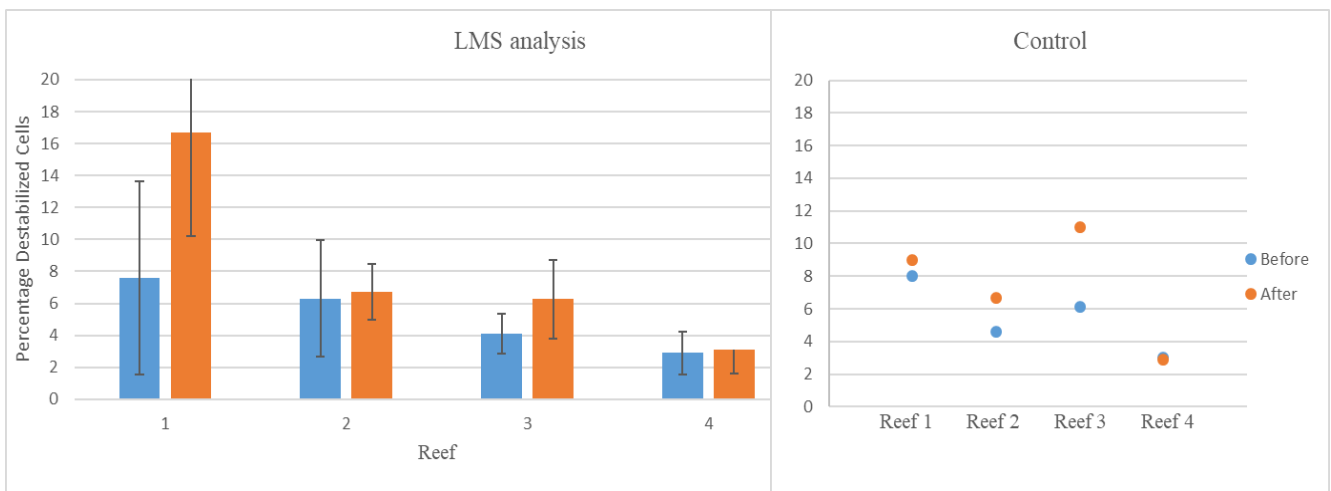
We calculated the relative respiration of coral fragments by dividing oxygen consumption at any given temperature by the oxygen consumption at the starting temperature of 5 °C for that same coral fragments. Results of the relative respiration of the coral fragments at all four reefs are presented in figure 13. At each reef, the colony designated by the letter B represents the corals kept in the control tank. This allowed us to investigate any differences caused through a time effect during the experiment duration.

Although further processing of the analyses is required, Fig. 13 already illustrates different sensitivities to temperature stress between different reefs as evident by the different slopes. Colonies from the Sula Reef (most off-shore region) had up to 8 times higher respiration rates at 15 °C than at 5 °C, while this value did not exceed 5 times in the other reefs. Each reef also displays different variation in respiration increase between its colonies, with Hola reef corals for example having much less total variation than the other reefs. Each reef also displays different variation in respiration increase between its replicates (branches from different colonies), with Hola reef for example having much less total variation than the other reefs.



**Fig. 13:** Respiration of coral branches from four different reefs relative to their starting respiration at 5 °C with increasing temperature.

Results of the LMS analysis showed a slight increase (0 to 2 times higher) in destabilised cells for corals subjected to temperature stress (Fig. 14), showing that corals are also effected on the cellular level under thermal stress.



**Fig. 14:** LMS-ratio for coral fragments in all four reefs, displayed as the percentage of destabilised cells before (blue) and after (orange) temperature stress (left panel). The control fragments (right panel) were not subjected to thermal stress for the experiment duration.

#### 4.4 Release and degradation of particulate and organic matter by *L. pertusa*

(Christine Ferrier-Pagès, Magali Boussion)

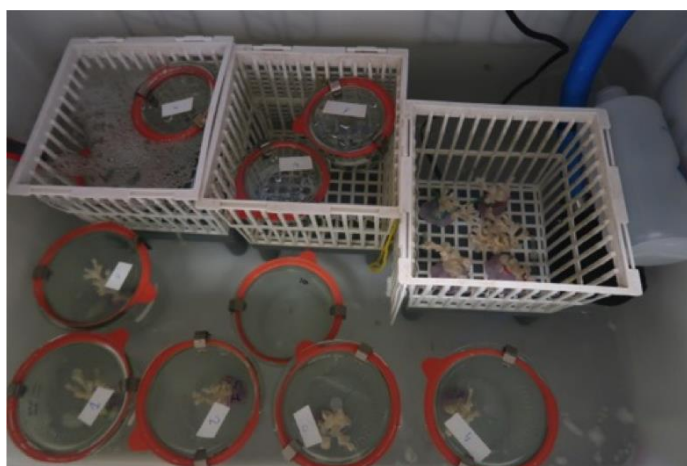
##### Goals

Scleractinian cold-water corals (CWC) represent key taxa controlling deep-sea reef ecosystem functioning by providing structurally complex habitats to a high associated biodiversity, and by fuelling biogeochemical cycles via the release of organic matter. Nevertheless, our current knowledge on basic CWC properties, such as feeding ecology and key physiological processes (i.e. respiration, calcification and organic matter release), is still very limited. During this field cruise, and in agreement with the other measurements performed by GEOMAR and the POS525 teams, we were in charge of assessing release and degradation rates of particulate and dissolved organic matter by colonies of *Lophelia pertusa* sampled in four different locations of the Norwegian coastal and offshore waters. We also collected and preserved colonies for later determination of total protein, lipid and carbohydrate contents to monitor the effects of different environmental conditions on the tissue energetic reserves of these cold-water corals. Finally, the last goal was to perform feeding experiments with  $^{13}\text{C}$  and  $^{15}\text{N}$  labelled *Artemia sp.* nauplii.

##### Measurements and methods

##### **Organic carbon and nitrogen fluxes**

After collection, colonies of *L. pertusa* were divided into small nubbins of few polyps and maintained one to two days in controlled aquaria on board of the vessel to recover from collection stresses. Organic matter release rates were then carried out by closed-cell incubation in acrylic respiration chambers placed in a temperature-controlled water bath (N=6; volume 240 mL, water-jacketed). Coral nubbins were transferred individually without aerial exposure inside a pre-rinsed (3 times with natural seawater) incubation chamber filled with  $0.45\ \mu\text{m}$  pre-filtered seawater (Fig. 15). A control chamber, filled only with pre-filtered seawater, was also prepared. The incubation lasted for 6 hours at a chamber temperature of  $12.0 \pm 0.1\ ^\circ\text{C}$ . Organic matter release will be assessed by calculating the difference in the seawater total organic carbon (TOC) and total nitrogen (TN) concentration between the beginning and end of the incubation (Naumann et al. 2010). Seawater samples (60 mL) were thus drawn before and after incubation from each jar and transferred into glass vials. All materials used for the sampling were cleaned from organic matter, and rinsed 3 times with distilled water, before being burned at  $480\ ^\circ\text{C}$  during 6 hours in a furnace prior to the cruise.



**Fig. 15:** Individual incubation jars for organic matter flux measurements on board RV POSEIDON. Photo: Magali Boussion.

The seawater samples were acidified with phosphoric acid (20 %, 250  $\mu\text{L}$ ) to  $\text{pH} < 2$ , and kept frozen ( $-20\text{ }^{\circ}\text{C}$ ) until analysis by high temperature catalytic oxidation (Shimadzu TOC-VCPH, Kyoto, Japan). Variation in the TOC and TN measured from the control jar will be subtracted from those measured in the coral jars, and TOC net fluxes will be derived from the recorded variation of TOC over the 6 hours incubation. Negative fluxes (from seawater to corals) will represent an uptake of organic matter by the coral colonies, while positive fluxes (from corals to seawater) will represent a release of organic matter. Fluxes will be normalized to skeletal surface area of the coral colony, determined by advanced geometric techniques according to Naumann et al. (2009).

### Organic matter degradation - mucus experiments

Understanding the release and recycling of organic matter by cold-water corals provides insight into biogeochemical cycling in deep reef environments. Micro-organisms are the major players of this organic matter recycling, through their exo-enzymatic activities.

The trophic link between heterotrophic bacteria-organic matter and higher trophic levels has been described as the microbial loop, which is at the base of the marine food web.

To collect freshly produced mucus, corals were removed from water immediately after collection and exposed to air for 15 minutes. Mucus produced was retrieved with filtered seawater by washing the surface of the coral colonies. The mucus obtained was then incubated for 48 to 72 hours and samples for TOC and TN were sampled regularly to assess changes in the concentrations over time. In addition, 1 mL was sampled at each sampling time and preserved in formaldehyde for the determination of bacterial concentration.

### Feeding experiments

To determine the feeding rates and incorporation of prey carbon and nitrogen by *L. pertusa*,  $^{13}\text{C}$  and  $^{15}\text{N}$  artemia prey were prepared, frozen and brought on the vessel for feeding experiments. Unfortunately, only three small colonies opened the polyps to perform those experiments and thus could be investigated.

The heterotrophic carbon (HC) and nitrogen (HN) incorporation in the coral colonies was obtained using  $^{13}\text{C}/^{15}\text{N}$ -labeled *Artemia sp* prey. For this purpose, a large batch of the microalgae *Dunaliella sp.* was grown in a Conway medium enriched with 2  $\text{mmol L}^{-1}$  of  $\text{NaH}_2^{13}\text{CO}_3$  (98 atom %  $^{13}\text{C}$ , Sigma-Aldrich, St Louis, MO, USA) and 1  $\text{mmol L}^{-1}$  of  $^{15}\text{NH}_4\text{Cl}$  (98 atom %  $^{15}\text{N}$ , Sigma-Aldrich). After being hatched, a large batch of *Artemia* nauplii was grown for 2 days in the  $^{13}\text{C}/^{15}\text{N}$ -labelled culture of *Dunaliella sp.* The labelled *Artemia* were then isolated by filtration on a 20  $\mu\text{m}$  mesh, divided into equal portions and frozen at  $-20\text{ }^{\circ}\text{C}$ . The *Artemia* prey was then given to the three coral colonies on board the RV POSEIDON once a day for three days. Afterwards, the fed coral fragments were frozen until subsequent analysis. The coral tissue will be detached from the skeleton using an airbrush in 0.45  $\mu\text{m}$ -filtered seawater. The slurry will be homogenised using a potter tissue grinder. Samples will be flash-frozen in liquid nitrogen and freeze-dried until analysis. The %  $^{13}\text{C}$ , and %  $^{15}\text{N}$ , as well as the carbon and nitrogen content of the animal tissue will be determined with a mass spectrometer (Delta Plus, Thermofisher Scientific, Bremen, Germany) coupled via a type III interface with a C/N Analyzer (Flash EA, Thermofisher Scientific).

### Sampling of coral fragments for total proteins, lipids, and carbohydrates

**Protein biomass** will be estimated using a bicinchoninic acid protein assay (Uptima, Interchim, Montluçon, Allier, France). For each sample, protein will be extracted by incubation in a sodium hydroxide solution maintained in a water-bath for 30 min at 90 °C. Subsequently, samples will be transferred into 96-well microplates, and incubated with a dye reagent (Uptima Reagents, Interchim) for 30 minutes at 60 °C. Protein standards across a concentration range from 0 to 2000 µg mL<sup>-1</sup> will be prepared using bovine serum albumin (BSA, Interchim). Samples and standards will be homogenised for 30 seconds at 400 r.p.m. on a microplate shaker. Absorbance will be measured at 560 nm, and sample protein content determined according to the standards. Data will be normalised to the skeletal surface area of the nubbins.

**Lipid content** will be quantified using a modification of the method developed by Bligh and Dyer (Bligh and Dyer, 1959). Frozen nubbins will be ground into a fine powder using a mortar and pestle and mixed with a solution of dichloromethane, methanol and distilled water (3.0 mL, 1.5 mL and 1.2 mL, respectively). Samples will be sonicated for 10 minutes, incubated at 40 °C for 1 hour and subsequently filtered through Whatman GF/C filters to remove skeleton fragments from solution. Afterwards, 1.5 mL of both dichloromethane and methanol will be added to the filtrate and the solution centrifuged at 2000 r.p.m. for 10 min. to separate the phases. The upper (methanol and water) phase will be removed and the lower (lipid containing) layer will be transferred into cleaned, pre-combusted and pre-weighed glass vials (4 mL). The solution will be evaporated under nitrogen, and the remaining amount of lipid determined by weight.

For **carbohydrates**, the protocol remains to be determined.

## 4.5 Sampling of coral fragments for reproduction, fatty acid, and genetic analyses

(Sandra Brooke, Janina Büscher)

### Goals

#### **Stony coral reproduction**

The objective of this study component is to assess the reproductive status of two species of stony corals (*Lophelia pertusa* and *Madrepora oculata*) from the study sites, and determine whether there are site-specific differences in fecundity and gametogenic condition. Under stressful or food-limited conditions, reproductive output may decrease as the coral shifts energy from gamete production to maintenance or growth, so assessing reproductive status will provide insight into whether some corals are exposed to sub-optimal conditions.

#### **Population genetic analysis**

The objective of this component is to assess the genetic structure of *Lophelia pertusa* populations from the different study sites. Understanding how closely populations are linked will provide insight into connectivity among fjord and offshore populations, and across different latitudes. This

information is important as it potentially identifies source and sink populations, which has implications for management.

### Fatty acids and lipid composition

Fatty acids and other lipids are biomolecules and play a crucial role in marine organisms, as they comprise dietary sources for animals, provide energy functions, regulate the metabolism as hormones, and are important structural components of cells, e.g. in bio-membranes (e.g. Eckert, 2002). In corals, lipids are also components of the mucus, which is important for catching prey and organic matter excretion as well as protecting the corals against environmental stresses like sedimentation (e.g. Mortensen, 2001). Analysis of the total amount of lipids and the composition of different lipid classes and fatty acids will give us insights into the health status and general physiological condition of *L. pertusa* and if this varies between the different locations as well as in- and offshore.

### Measurements and methods

#### Stony coral reproduction

Samples of stony corals were collected from each of the four study sites (table 2) using the JAGO manipulator arm. Each sample comprised one or more small branches containing approximately 10 polyps, which were placed in 10 % fully buffered formalin and processed using standard histological techniques (e.g. Brooke and Järnegen 2013).

**Table 2:** Summary of samples collected for reproduction, genetic and fatty acid (lipid) analysis.

| Species                  | Site Name  | Depth (m) | Date                                      | Samples |
|--------------------------|------------|-----------|---|---------|
| <i>Lophelia pertusa</i>  | Hola       | 250-260   | 11-14 <sup>th</sup> July                  | 14      |
| <i>Lophelia pertusa</i>  | Nord-Leksa | 165       | 5-6 <sup>th</sup> July                    | 9       |
| <i>Lophelia pertusa</i>  | Steinavaer | 205-250   | 8-9 <sup>th</sup> July                    | 19      |
| <i>Lophelia pertusa</i>  | Sula       | 278-302   | 1-2 <sup>nd</sup> , 16 <sup>th</sup> July | 15      |
| <i>Madrepora oculata</i> | Steinavaer | 244       | 8 <sup>th</sup> July                      | 1       |
| <i>Madrepora oculata</i> | Sula       | 281-302   | 2 <sup>nd</sup> , 16 <sup>th</sup> July   | 5       |

Prior to histological processing, the calcified skeleton of each sample will be dissolved in 10 % hydrochloric acid, then polyps will be rinsed in distilled water and dehydrated through a series of ethanol concentrations (70 %, 80 %, 95 %, and 100 %). The tissues will be cleared overnight using HistoClear, embedded in paraffin wax, then sliced into 8-µm sections using a Leica microtome. Sections will be mounted onto microscope slides, dried for 12 hours, and stained using Mayer's Haematoxylin (which stains DNA dark blue) and Eosin B (which stains cytoplasmic proteins bright red). After staining, the sections will be mounted and left to dry. Sequential images of all the sections will be taken using an Optronics digital camera attached to an Olympus BX50 compound microscope. For each female fragment, 100 oocytes will be measured from 5-10 polyps. Only those oocytes with visible nucleoli will be measured. This ensures that the same egg is not measured more than once, as the nucleolus is so small (approximately 9 µm diameter) that it only appears in one 8-µm slice. The oocyte area will be measured and recorded using Digimizer image analysis software. Fecundity will be assessed by counting oocytes within serial sections. As oocytes are usually not



completely circular, oocyte “feret” diameter will be calculated using the following formula, which estimates the diameter of a hypothetical circle with the same area as the object measured.

$$\text{Ferret diameter} = \frac{\sqrt{4 \times \text{area}}}{\pi}$$

Oocyte diameters and size-frequency distributions will be generated for each female, and combined by site to infer the timing and periodicity of female gametogenic cycles. The developmental stage of each female will be assessed using criteria described in Waller and Tyler (2005) as follows:

- Stage I: Oogonia are visible in the mesenterial lamellae;
- Stage II: Pre-vitellogenic oocytes are small with thin wall and basophilic cytoplasm;
- Stage III: Early vitellogenic oocytes with a small amount of cytoplasm;
- Stage IV: Late vitellogenic oocytes with granulated cytoplasm and thick cortical periphery;
- Stage V: Post spawning, with some remaining eggs. Where there is sufficient data, oocyte diameter will be compared between sampling months and years using a student’s t-test.

Male reproductive maturity will be documented qualitatively by developmental stage, as size of spermatocysts can vary greatly within a single developmental stage, unlike oocytes, which increase in size as they develop. Male gametogenic cycles will be documented by stages as follows:

- Stage I (early spermatogenesis): Spermatocysts are lined with spermatocytes but lumens are empty;
- Stage II (maturation phase): Thick layer of spermatocytes with some spermatozoa present, but with mostly empty lumens;
- Stage III (mature): Spermatocyst lumens are filled with spermatozoa;
- Stage IV (post spawn): Spermatocysts are empty of spermatozoa, except occasional remnants of spawning (Waller & Tyler 2005, Brooke & Järnegren 2013).

Oocyte diameter, reproductive stage, and fecundity data will be compared across sites using Analysis of Variance (ANOVA) to compare average values within each site with those from other locations. These data will not only address reproductive biology at the project study sites, but will also contribute to an ongoing study of the timing of *Lophelia pertusa* reproduction from the eastern and western Atlantic and Gulf of Mexico.

### Population genetic analysis

For each of the samples collected for reproduction (table 2), a second branch was preserved in molecular grade ethanol for genetic analysis. Genetic samples will be processed by Dr. Morrison from the US Geological Survey, using methods described in Morrison et al. (2011). DNA will be extracted and microsatellite loci will be amplified using PCR (Morrison et al. 2008). Within-site genetic diversity will be assessed by comparing observed and expected heterozygosities under Hardy–Weinberg equilibrium (HWE), and calculating fixation indices per microsatellite locus and study site. Genetic relationships among populations and individuals will be estimated from the microsatellite data using several approaches as outlined in Morrison et al. 2011. These will include model-based clustering, calculation of pairwise genetic distances between sites, analysis of molecular variance and isolation by distance calculations. These data will not only address

population structure and connectivity among the study sites, but will also contribute to an ongoing comprehensive analysis of Atlantic populations of *L. pertusa*.

### **Fatty acids and lipid composition**

For each of the samples collected for reproduction and genetics listed in table 2, also a third fragment à 5-10 polyps was taken from the same colony like the previous samples and preserved immediately by being frozen in liquid nitrogen (LN<sub>2</sub>, -196 °C). Afterwards, the samples were kept frozen in a -80 °C freezer until end of the cruise, after which they were transferred to -20 °C at which they will be kept until analysis.

### Preliminary results

There has been no progress on this objective as the samples are at GEOMAR awaiting shipment to the partner institutes in the US or processing in own laboratories in case of fatty acid analyses. Since stony corals are CITES II listed species, both institutions, the sender as well as the receiver institute, require CITES permits in order to transfer the samples. Progress is being made towards obtaining these permits.

## **4.6 Reef community analysis**

(Sandra Brooke)

### Goals

The overall aim of this project component was to characterize the different study sites in terms of percent live cover of *Lophelia pertusa*, and diversity and abundance of associates. Understanding the 'condition' of each study area will provide context for the biological observations from the other project components.

### Measurements and methods

#### *Video analysis*

A Canon High definition (HD) digital camera was mounted inside the submersible and recorded video continuously while on bottom. The JAGO navigation files (including latitude, longitude and depth) and the dive videos will be synchronized via their time codes so that position and depth data can be assigned to observations on the video. Video will only be analysed when the vehicle is transiting, with lasers on and with adequate visibility to enable habitat and faunal descriptions. All poor quality video and sections where the JAGO is stationary will be removed. The remainder of the video will be categorized into one of four geological habitat types, which encompasses the dominant combinations of soft and hard substrates, including percentage of dead coral matrix. In addition, the percentage of live *Lophelia* will be determined and combined with the geological habitat category during analysis (table 3).

To generate uniform-sized replicates, video of each habitat category will be split into 15-second intervals. For each time interval, all corals and large sessile (sponges, hydrozoans, anemones etc.), and mobile (fishes, crabs, etc.) organisms will be identified and counted. Communities associated with each habitat type and study site will be analysed using multivariate statistics (Primer-E V6 software; Clarke & Gorley 2006) to determine whether there are significant differences between factors (study site and habitat type). Prior to analysis, an appropriate transform will be applied to the frequency data. Bray-Curtis similarity matrices will be created and multi-dimensional scaling (MDS) plots generated for by each factor. A two-way analysis of similarity (ANOSIM) will be used to analyse differences in community structure by habitat type, nested within site (see Brooke et al., 2017).

#### *Transect analysis*

Of the 22 submersible dives, seven (JAGO 2, 7, 11, 12, 16, 17, 22) had a Go-Pro camera mounted on the outside in a downward-looking position, which is ideal for quantifying percent cover or abundance within a known and consistent area. Transect images will be analysed for these seven dives, in addition to the video analysis. This will enable a useful comparison of techniques. Non-overlapping images will be selected for analysis using Point Count for Corals (CPCe; National Coral Reef Institute). This software provides a tool for the determination of benthic cover using a specified number of spatially random points, which are distributed on a transect image. The features (geological and biological) below each point are identified manually, and the coverage statistics for each frame and transect are calculated by the software (Kohler & Gill 2006). Community data will be analysed using Primer-E V6 software in the same way as for the video analysis.

**Table 3:** Habitat categories used for community analysis.

| Habitat Type                        |   |
|-------------------------------------|---|
| <b>S</b>                            | Sediment: Soft substrate with no hard substrate visible.                        |
| <b>LR</b>                           | Low Rubble: Sediment with < 25 % cover of small pieces of rock or coral rubble. |
| <b>MR</b>                           | Moderate Rubble: Sediment with 25-75 % cover of rock or coral rubble/framework  |
| <b>HR</b>                           | High Rubble: Sediment with > 75 % cover of rock or coral rubble/framework       |
| Percentage of live coral categories |   |
| <b>1</b>                            | 0   |
| <b>2</b>                            | < 25 % cover  |
| <b>3</b>                            | 25-75 % cover   |
| <b>4</b>                            | > 75 % cover  |

#### Preliminary results

The pre-processing of digital imagery was initiated at the start of the FSU fall semester (August 27<sup>th</sup>), with the assistance of an FSU undergraduate Honors Student. Unusable video has been removed from 16 of the 22 dives, in preparation for habitat analysis. Brooke will train the student in video processing and will conduct quality assurance checks to ensure data accuracy. By the end of this semester, the video data extraction will be completed. If time permits, the student will begin work on the transect images. Brooke will be responsible for the statistical analysis of both video and transect data.

## 4.7 Isotope measurements and proxy calibration analysis

(Nico Schleinkofer)

### Goals

The main purpose of the samples is to get *in situ* data from water samples in combination with adjacent samples of the bivalve *Acesta excavata*. This data will be used for proxy calibration (i.e. Mg/Ca, Sr/Ca,  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ ). Especially oxygen isotope measurements of the water are necessary to achieve a good calibration, since the isotopic composition of the water must be known. Elemental concentrations of the water are needed to research in which way the elemental composition of the ambient water alters the elemental composition of the bivalve shells. Additional information about biomineralisation models can be obtained (e.g. Rayleigh Fractionation, ion pathways).

### Measurements and methods

#### **Water sampling**

A Water Sampler Rosette / CTD (conductivity, temperature, depth) system (details in section 5.2) was used to study water chemistry and hydrography in the research area (see also 4.8 for water column characterisation). In each of the four reefs, at least three CTD casts for sampling were conducted. These three casts were distributed to form a profile parallel to the main current of the reef with one cast on the reef top and one cast on the luv and lee side of the reef, respectively. For each cast three water depths were sampled, surface water, bottom water and the thermocline. Additional casts were conducted in consideration of the scheduled JAGO dives.

#### **Sample preparation**

After CTD recovery the water samples were transferred to sample bottles and treated according to their intended purpose described in the following:

##### *Element concentrations*

Water samples were filtered through a 0.45  $\mu\text{m}$  Millipore® cellulose acetate filters and transferred to 25 ml pre-cleaned (10 %  $\text{HNO}_3$ ) Nalgene Bottles. Before sampling the bottles were rinsed with sample water. The samples were acidified with 40  $\mu\text{L}$  of 69 %  $\text{HNO}_3$  to have  $\text{pH} < 2$  and keep the ions from precipitating. The samples were stored in a fridge for further measurements.

##### *Stable isotopes*

Water samples for stable isotope analyses were transferred to 100 ml borosilicate glass bottles. Before sampling the bottles were rinsed with the sample water. The bottles were rotated during filling and allowed to overflow to avoid air bubbles in the samples. The bottles were sealed with rubber stoppers and crimped with metal seals. A little headspace (few mL) was allowed to prevent the bottles from popping the seal when frozen. The samples were preserved by adding 100  $\mu\text{L}$   $\text{HgCl}_2$  and stored in a fridge.

### **Acesta sampling**

Specimens of *Acesta excavata* were collected alive with the JAGO submersible where they were found, i.e. mainly in the fjord reefs at Nord-Leksa and Steinavaer. In Høla, no *Acesta* sp. were found and in Sula, some *Acesta* were spotted, but could not be retrieved as they were hidden in the dense crest in the top reef colonies. The bivalves were placed alive in a cultivation tank filled with (deep) seawater, which was kept at a constant temperature until further processing (see 5.4). For further sampling, the clams were cut open at the abductor muscle and the gills, muscles and other tissue were separated, stored in plastic tubes and frozen in liquid nitrogen. The shells were cleaned from any remaining tissue material and dried in an oven. Shells will be used for elemental concentration and stable isotope measurements at Goethe University Frankfurt.

### Preliminary results

All taken samples are awaiting analysis at the department of Geosciences at the Goethe University in Frankfurt. Thus, no results can be presented for the time being.

## **4.8 Water column characterisation**

(Nico Schleinkofer, Janina Büscher)

### Goals

In order to describe the different habitats of the spatially distinct cold-water coral occurrences, the environmental factors such as the physicochemical parameters temperature, salinity, and the carbonate chemistry have to be investigated. For this, at all stations CTD casts were carried out several times during the day at different locations of the reefs (top and the flanks). Thereby, temperature, salinity, oxygen concentration and fluorescence as an indicator of plankton density were measured among others by means of sensors attached to the CTD frame (see 5.2), while the carbonate chemistry will be analysed from water samples taken at different depths of the water columns.

### Measurements and methods

For the details of the CTD instruments the reader is referred to chapter 5.2, where the description of the CTD parameters can be found.

The profiles of the CTD casts were plotted with respect to their different locations and are described in the following results section.

Water samples for dissolved inorganic carbon, total alkalinity and dissolved inorganic nutrients were taken in the same rhythmicity during the same CTD casts and at the same depths as the samples for stable isotopes (see 4.7).

*Dissolved Inorganic Nutrients*

Samples for dissolved inorganic nutrient (nitrate, silicate, phosphate, and ammonium) measurements were drawn directly from the NISKIN bottles after rinsing the vials. Samples were either taken in 100 mL glass vials or PE vials (Nalgene®) and stored at -20 °C for later analysis.

*Total Alkalinity (TA)*

Water samples for TA were transferred from the NISKIN bottles to 100 ml PE vials (Nalgene®) after rinsing. The samples were preserved by adding 100 µl HgCl<sub>2</sub> and stored in a fridge. Measurements will be conducted at GEOMAR, Kiel using standard titration techniques (open-cell grand titration).

*Dissolved Inorganic Carbon (DIC)*

DIC samples were drawn directly from the NISKIN bottles into rinsed 50 ml borosilicate glass bottles. Bottles were rotated during sampling and slowly filled and closed after being allowed to overflow to for a while in order to prevent air bubbles. Afterwards, samples were poisoned with 100 µL HgCl<sub>2</sub> and stored in a fridge.

All water samples remain to be analysed yet and the preliminary results refer to the CTD cast profiles only.

Preliminary results

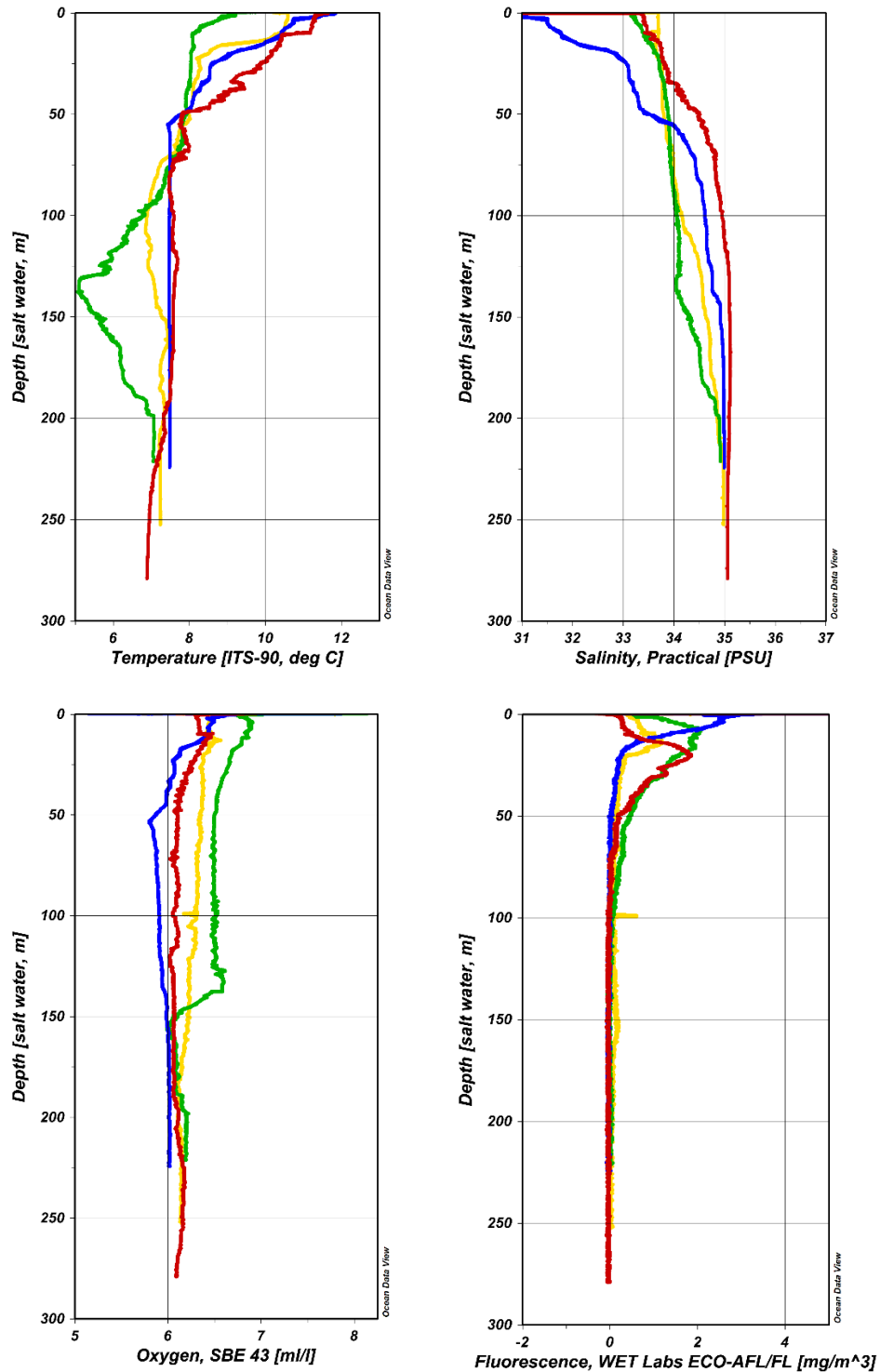
The temperature profiles of Nord-Leksa, Sula and Hola were rather uniform with surface temperatures between 10 and 12 °C and a well-developed thermocline between 10 – 50 m (Fig. 16). In Nord-Leksa the temperature was very stable from 50 m downwards and amounts to 7.48°C. Deep-water temperatures in Sula and Hola were much more variable but stabilised below 200 m at 6.9°C and 7.24 °C, respectively. The temperature profile from Steinavaer shows a minimum temperature of 5.1 °C at 150 m depth and increasing temperatures at higher depth up to 200 m, where the temperature stabilised at 7.07°C. The same maximum is visible in the temperature profile from Hola but not as pronounced. Possible reasons include cascading water masses or the occurrence of different water masses in the Hola / Steinavaer area.

Salinity profiles are uniform for the four reefs except for the Nord-Leksa reef, which shows fresher surface water than the other reefs (Fig. 16). Sula and Nord-Leksa were more saline in depths from 50 to 200 m than Steinavaer and Hola. Below 200 m the salinity at all reefs stabilised around 35.

The distribution is similar in the Fluorescence profiles (Fig. 16). Hola, Steinavaer and Sula showed maximum fluorescence at 20 – 30 m water depth, whereas Nord-Leksa showed the highest fluorescence at the surface. Below 70 m the fluorescence stabilised around 0 mg/m<sup>3</sup>. Only in Hola reef, a sharp peak at 100 m was visible.

Oxygen concentration was uniform in all four reefs and varied between 5.8 and 7 ml/L (Fig. 16). Hola and Steinavaer had slightly increased oxygen concentrations in comparison to Sula and Nord-Leksa. The differences between Sula / Nord-Leksa and Hola / Steinavaer in temperature, salinity and oxygen concentration can be explained by the influence of Winter Mode Water (WMW) at the northern Hola / Steinavaer reef (Rüggeberg et al., 2011). At Hola reef an oxygen minimum was

recognisable at 100 m water depth. This can be caused by higher planktonic activity in this depth, which would also explain the high fluorescence. Below 150 m all reefs had stable oxygen concentrations of about 6 mg/L.



**Fig. 16:** Exemplary results of the CTD casts plotted for temperature vs. depth (upper left), salinity vs. depth (upper right), oxygen vs. depth (bottom left), and fluorescence vs. depth (bottom right) at the different reef locations Nord-Leksa (blue, CTD\_010), Sula (red, CTD\_013), Steinavaer (green, CTD\_021), and Hola (yellow, CTD\_021).

## 5. Scientific equipment and instruments

### 5.1 Submersible JAGO

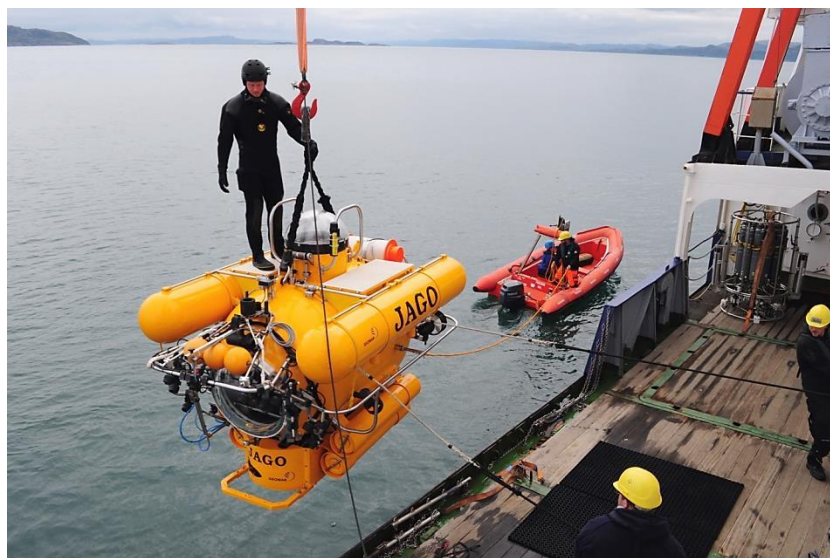
(Karen Hissmann, Jürgen Schauer, Peter Striewski)

#### System description and application during POS525

The largest research gear used during POS525 was the GEOMAR-owned manned submersible JAGO (Fig. 17) that can take two persons – a pilot and a scientific observer – to water depths of 400 m maximum (Hissmann & Schauer, 2017). The submersible has a compact size and a low weight of 3 tons that enables shipment in a single 20' ISO container and deployment from a wide variety of support vessels that have sufficient crane capacity. JAGO is equipped with USBL navigation and positioning system for tracking the submersible under water, compass, vertical and horizontal sonar, underwater acoustic telephone for voice communication (Subphone 580 by Subsea Import), LED lamps, digital video (HD) and still cameras, CTD sensors and a manipulator arm for collecting and handling various sampling devices and instruments.

The submersible operates worldwide and is regularly used from the German research vessels including RV POSEIDON. The POSEIDON is particularly suitable for handling JAGO, since it has a low working deck of only 1.5 meters, which facilitates short deployment and recover manoeuvres.

The submersible has been frequently used for cold-water coral research during previous cruises (POS228 (1997), POS253 (1999), POS325 (2005), POS391 (2009), POS420 (2011), POS455 (2013), POS473 (2014) with RV POSEIDON; AL275 (2006), AL290 (2008) with RV ALKOR; and PS ARK 22/1a (2007) with RV POLARSTERN).



**Fig. 17:** Submersible JAGO being deployed off deck of RV POSEIDON for an exploration dive at Nord-Leksa Reef, Trondheimsfjord, Norway. Picture: K. Hissmann.

The mobilisation of the submersible on board the POSEIDON took place from 27 to 28<sup>th</sup> June in the port of Bergen (installation of the USBL underwater navigation and positioning system, UT-communication, preparation and mounting of sampling devices etc.).



On deck, JAGO was lashed amidships, and lifted and transferred into and out of the water over the ship's side by the main deck crane (SWL 5 tons). The vessel's Rigid Inflatable Boat (RIB, 5.5 m TL, 60 HP Yamaha outboard engine), steered by a crew member, was used to tow the submersible away from the ship's side after deployment and back under crane position for recovery.

While submerged, JAGO was tracked by means of the USBL underwater positioning and navigation system ORE Trackpoint 3P (manufactured by ORE EdgeTech USA) that comprises, a transceiver and a transducer (mounted on a pole inside the moon pool of the vessel) as top side units, and a transponder attached to JAGO's top railing as bottom unit. The position data were integrated into the navigation software OFOP (<http://www.ofop-by-sams.eu/>) to display and follow both JAGO and POSEIDON tracks geographically and in real time on a computer screen. Position data were logged in column-based ASCII files. Dive tracks can be combined for annotation with individual dive logs, visual and video observations and CTD records. The recording time for all cameras and sensors was synchronised with the ship's clock and set to UTC.

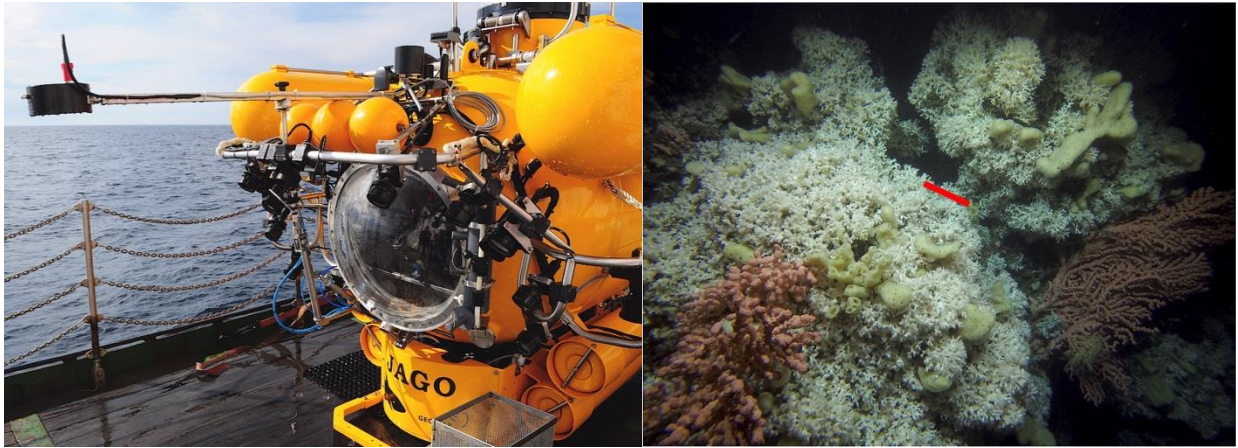
The water column and sea floor in front of the submersible's acrylic bow window, as well as all activities performed with the manipulator arm were continuously HD video-documented from inside the submersible with a CANON XA25 HD-Camcorder. The camera was mounted in the centre of the large acrylic bow window. The camera's 3.67-73.4 mm zoom lens allows wide-angle and detailed close up footage of any object and structure in front of the submersible. The footages were recorded directly from the camera sensor onto an external hard drive (Shogun from Atomos) in ProRes 422 LT format as .mov-file. After each dive, the original HD video files were copied onto a NAS-server for storage and further processing. Metadata, such as recording time in UTC, can be displayed by viewing the videos with Quicktime 7 or image annotation software like OFOP. In addition, a compressed copy of each video file was produced in H.264/MPEG-4 AVC format and overlaid with UTC time stamp for easy geo-referencing. Video still images can be captured from the original HD footage by frame grabbing. A dive protocol was written by the dive participants to note observations and activities during the course of the dive.

In addition to the Canon Camcorder for continuous HD video recording, a GoPro Hero4 mini-camera in an underwater pressure housing was used for time lapse photographing of the seafloor during some of the survey dives. The camera was mounted on a horizontal extension pole at the front of the submersible directed downwards (Fig. 18) to capture the seafloor for community analyses (see chapter 4.6). The GoPro was set to JPG still mode with an interval of 2 seconds, image size 12 Mpix, maximum wide angle, 6500 K, and spot light exposure measurement. Images were stored on an internal micro-SD card. The camera was power-supplied from inside the submersible.

For quantifying the size of objects observed on the seafloor and captured on video and still images, two green laser points were projected on the seafloor. The distance between the two parallel laser beams was 20 cm (Fig. 18).

A CTD (SAIV A/S SD204 Norway), attached to the stern of the submersible, continuously recorded depth, temperature, salinity and density during de- and ascents and while the submersible was at or close to the seafloor. The CTD data are available as ASCII files.

Water samples were collected close to the sea floor with a 2.5 litres NISKIN bottle attached to JAGO's front (partly visible in Fig. 19). The NISKIN is triggered with JAGO's manipulator arm.



**Fig. 18:** Submersible JAGO on deck of RV POSEIDON with external GoPro-mini-camera in underwater housing mounted on an extension pole (left), right: one of the still images taken during one of the survey dives at Nord-Leksa. Red bar indicates the distance between the two laser points (20 cm). Pictures: JAGO-Team.

JAGO was used to selectively collect fragile colonies of stony corals (*Lophelia pertusa*, *Madrepora oculata*), bivalves (*Acesta excavata*) and various species of reef-associated sponges with its minimal invasive manipulator arm (Fig. 19). Single branches of living corals - collected for genetic, reproduction and fatty acid analyses (chapter 4.5) – were stored in closable acrylic sampling tubes attached to the sampling box (Fig. 19, right, bottom left) in order to maintain those samples in their surrounding water.



**Fig. 19:** Stony coral samples collected with the sensitive claw of JAGO's manipulator arm (top left) and stored in the sampling box (right) or and in closable tubes to differentiate specific samples (i.e. white and red colonies from the same area; lower left). Pictures: JAGO Team.

For recovery of the automated incubation chambers (CUBE, see chapter 4.2) with JAGO, snap hooks were attached to the end of a rope that was tied to the lamp bar of the submersible. The rope was sufficiently long to guarantee that the hooked CUBE would hang below the submersible when lifted off from the bottom. The snap hook was hooked to the salvage ring on top of the chamber with JAGO's manipulator arm.

## 5.2 CTD (board CTD of RV POSEIDON)

(Nico Schleinkofer, Janina Büscher)

The SBE 9plus CTD unit from Seabird Electronics was mounted below the water sampler. Additionally, the CTD was equipped with a fluorescence sensor, two oxygen sensors and a turbidity sensor. Sampling rate of the sensors amounts to 24 Hz with 41 Bytes per scan. Data recording and NISKIN bottle triggering was controlled with SEASAVE software from a ship mounted computer. GPS position was logged from an NMEA-string of RV POSEIDON. The cast data was processed by using SEASAVE (V. 7.26.1.8) to convert the .hex data to .cnv data. Each cast was converted separately. In total 38 casts were conducted in four different reefs (8\*Nord-Leksa, 9\*Sula, 7\* Steinavaer, 14\*Hola). The system was controlled by winch 2 on portside of the ship. Depth control was achieved through pressure readings and an altimeter. The water sampler was equipped with 12 x 10 L NISKIN bottles. Example CTD profiles of this cruise are depicted in figure 16 in chapter 4.8 ("Water column characterisation"). Water samples taken during the cruise at all four stations are described in chapter 4.8, too, and are listed in the sample list in the appendix (chapter 9: C).

## 5.3 Water pump

(Janina Büscher)

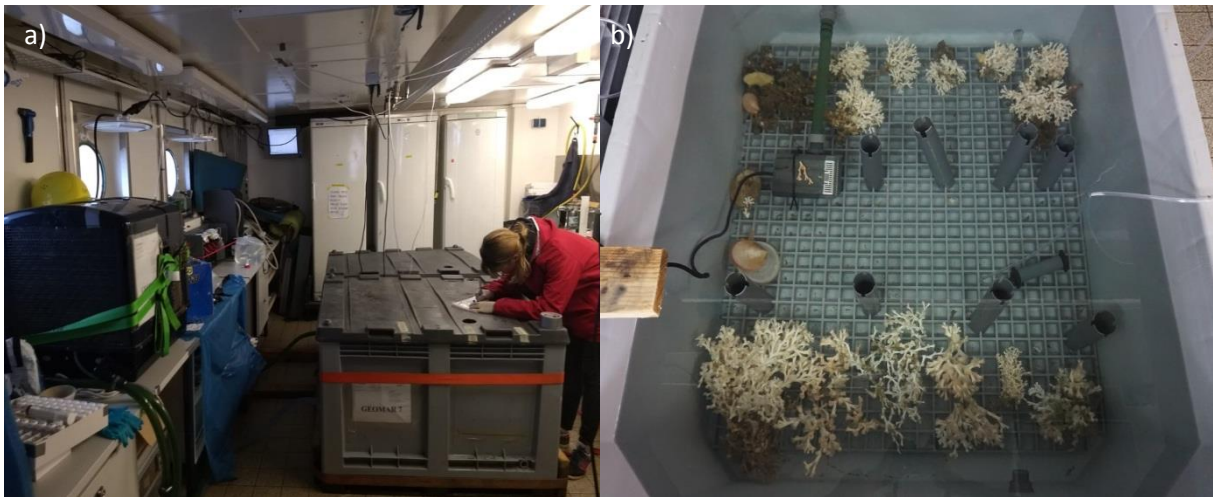
A submersible pump, typically used for rural sewage treatment, was applied for deep-water pumping by connecting a 100 m long hose (2.5 cm in diameter) to the pump with its lose end attached to the CTD frame. First, the lose hose end was lowered together with the CTD by connecting CTD wire and hose every 10-15 meters to provide stable pumping conditions. The underwater pump was attached to the hose and brought to the seawater surface, but lowered only 3-4 meters. The pump has an external power unit, which could be switched on and off on demand. Before filling the aquaristic tanks and containers, the pump and hoses were rinsed with the seawater pumped from about 100 m water depth (below the thermocline). The water was pumped with a continuous water stream at a rate of approximately 37.5 L/min. The water in the on-board cultivation facilities was exchanged regularly and the ship's own aquaria tanks were filled with offshore water from Sula and Hola as water reserves for on-board experiments and home laboratories.



## 5.4 Cultivation of living deep-sea organisms in large holding tanks

(Janina Büscher)

Live coral branches as well as dead coral framework including accompanying fauna were cultivated on board in two large interconnected tanks (PVC pallet boxes à 120 x 100 x 80 cm), recirculating a water volume of ca. 1500 L (Fig. 20), which was cooled to ambient seabed temperature of 7.5 °C ( $\pm 1$  °C) by means of a 4000 W cooling unit (Aqua Medic, Titan 4000; Fig. 20a) on the left). Two water pumps (EHEIM, Universal pump 1200) were installed, each in one cultivation tank, that circulated the water from one of the tanks to the cooling unit, further to the second tank and back to the first tank. Moreover, air stones were added to provide proper aeration in the tanks. The coral fragments were placed on a lattice (5 \* 5 cm grid size) to arrest them in a suitable position in the tank and avoid slipping during ship movement. The corals were brought in a plastic container inserted into the JAGO sampling container in front of JAGO before each dive to the wet lab of RV POSEIDON and inserted carefully into the cultivation tanks. The coral fragments were permanently kept under water if possible. Due to biological processes the water was exchanged every now and then to sustain water quality with fresh deep seawater from about 100 m water depth (see section 5.4).



**Fig. 20:** Cultivation set-up with two large PVC tanks installed and secured in the middle of the wet lab of RV POSEIDON and connected to a cooler (left, picture a)). b) View into one of the cultivation tanks after coral collection, equipped with a grid and PVC spacers for purposes of assignment of coral groups. Pictures: a) Peter Striewski, b) Janina Büscher.

## 6. Additional remarks

### 6.1 Media outreach activities

In addition to the scientific purposes, media activities were going on during the cruise. For one thing, we initiated a cruise blog on the platform 'oceanblogs.org' for the interested people to follow our activities during the cruise. The cruise blog for RV POSEIDON cruise 525 can be viewed on the following link:

<http://www.oceanblogs.org/pos525/>

Moreover, during the first few days of the cruise we had the opportunity to be followed by two camera operators from the 'Ocean Mind Foundation' in Kiel, who documented our research with a new 360°, 3D camera system with the purpose to give little children an understanding of how a JAGO dive and science on board a research vessel is being carried out. Especially kids in pre-school age, but also interested adults shall be introduced to the techniques of deep-sea research and experience a dive with the submersible through virtual reality visualisation.

### 6.2 CITES regulations

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an international agreement between governments with the aim to ensure that international trade in specimens of wild animals and plants does not threaten their survival. In Germany, the Federal Agency for Nature Conservation (Bundesamt für Naturschutz, BfN) is the management authority for CITES regulations. In Norway, the access authority is the Norwegian Environment Agency (Miljø-Direktoratet). Scleractinian corals (i.e. *L. pertusa* and *M. oculata* in particular) are recorded among the Appendix II-listed species of potentially endangered species. In the following table (table 4), the coral samples of collected species collected at the different sites during POS525 in July 2018 are listed. The amount in weight of the collected material is a rough estimate including not only living coral fragments, but also dead coral framework.

**Table 4:** List of cold-water coral samples collected at the various stations during POS525 cruise with the aid of the manned submersible JAGO.

| Station       | Species                  | JAGO Dive #   | Sample  | Estimated amount (kg) |
|---------------|--------------------------|---------------|---|-----------------------|
| I Sula        | <i>Lophelia pertusa</i>  | 4, 19         | Colonies of white and red colour morphs, and dead framework | 8                     |
| I Sula        | <i>Madrepora oculata</i> | 4, 19         | Two white and one red colony                                | 0.2                   |
| II Nord-Leksa | <i>Lophelia pertusa</i>  | 8             | Colonies of white and red colour morphs, and dead framework | 5                     |
| III Hola      | <i>Lophelia pertusa</i>  | 13, 16, 17    | Colonies of white and red colour morphs, and dead framework | 5                     |
| IV Steinavaer | <i>Lophelia pertusa</i>  | 9, 10, 11, 12 | Colonies of white and red colour morphs, and dead framework | 7                     |

## 7. Acknowledgements

The scientific party of the POS525 cruise gratefully acknowledges the very good cooperation and technical assistance of both captains and the entire crew of RV POSEIDON, who substantially contributed to the overall success of this expedition. The Technology and Logistics Centre (TLZ) at GEOMAR is thanked for the excellent logistic support and GEOMAR Helmholtz Centre for Ocean Research in general for financial support enabling equipment transport for this cruise, as well as employment of submersible JAGO. We acknowledge the support and the research permits issued by Norwegian Joint Headquarters, the Federal Foreign Office/German Embassy Oslo, and The Directorate of Fisheries. We are also grateful for the CITES permits given by the Norwegian Environment Agency for export (Ref. No. 18NO-009-EX) and by the Federal Agency for Nature Conservation for import (Ref. No. E-03456/18). Moreover, we appreciate the MAREANO project for the access of detailed bathymetric maps, which are very useful for conducting the submersible dives. We thank Briese Research for their support and immediate response after consultation. We are truly grateful to Neptune and Poseidon for the very favourable weather conditions and calm sea!

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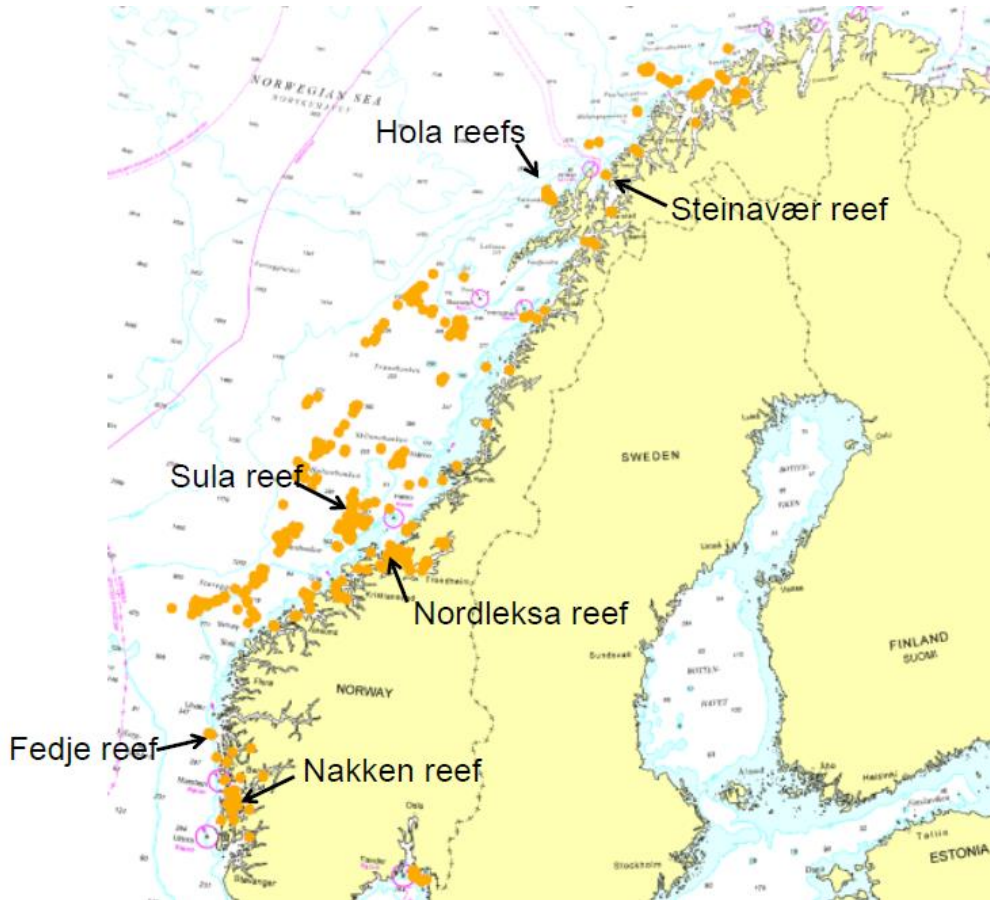
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## 9. Appendices

### A. Maps of cruise stations



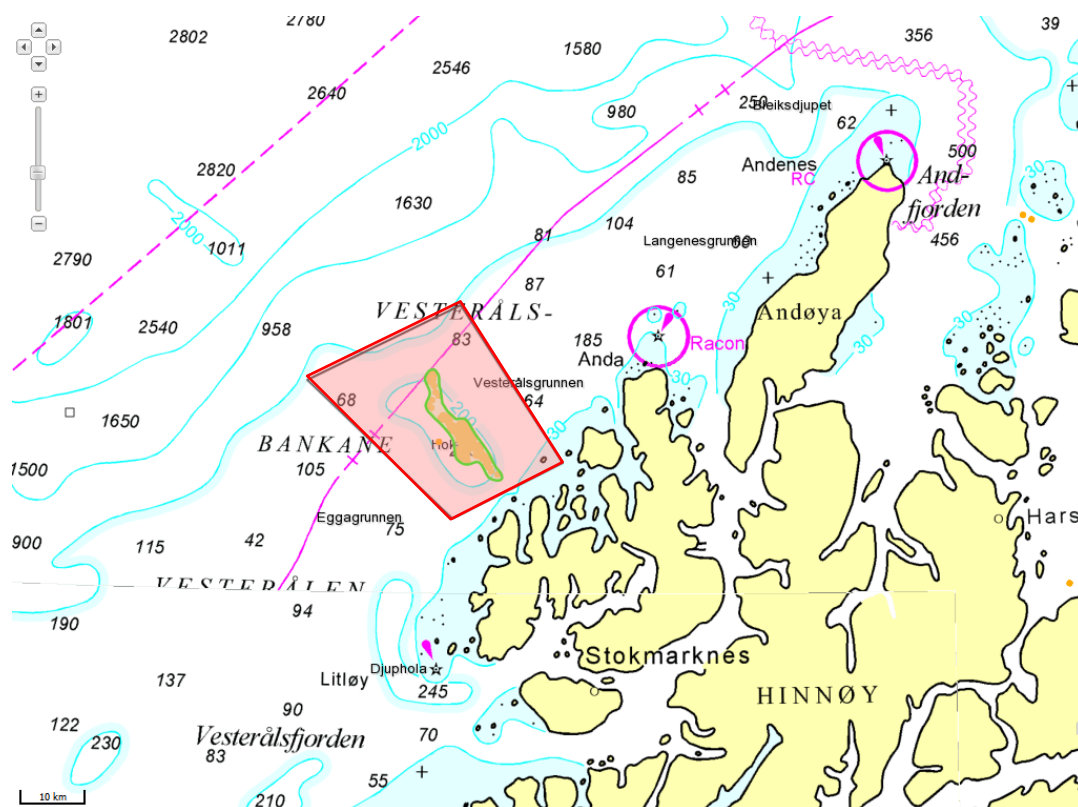
**Fig. A1:** Map of Norway displaying an overview of all six stations considered at the time of applying for the ship time for the cruise with RV POSEIDON along the Norwegian coasts and margins, including the actually approached reef sites Sula, Nord-Leksa, Holo, and Steinavaer.

**Table A1:** Coordinates of the four different research areas depicted in detail in the following maps (A2-A5).

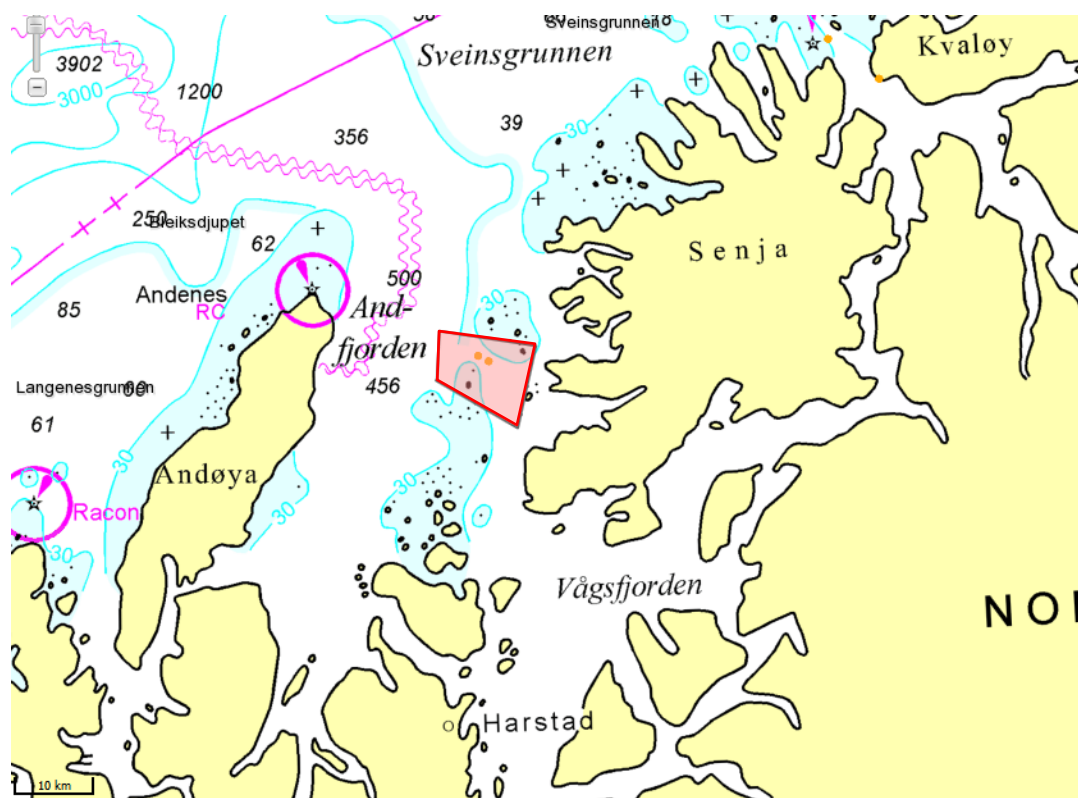
| Station | Time of stay                                 | Name             | Latitude (N) | Longitude (E) |
|---------|--|------------------|--------------|---------------|
| I       | 01.07.18 - 03.07.18<br>+ 16.07.18 - 18.07.18 | Sula reef N      | 64°17'55.1"  | 07°53'07.9"   |
|         |  | Sula reef E      | 64°10'32.5"  | 08°17'04.2"   |
|         |  | Sula reef S      | 63°52'29.0"  | 07°51'43.0"   |
|         |  | Sula reef W      | 63°59'59.2"  | 07°26'25.2"   |
| II      | 04.07.18 - 06.07.18                          | Nordleksa reef N | 63°37'14.7"  | 09°24'27.9"   |
|         |  | Nordleksa reef E | 63°36'06.9"  | 09°24'30.4"   |
|         |  | Nordleksa reef S | 63°35'46.6"  | 09°21'42.7"   |
|         |  | Nordleksa reef W | 63°37'07.3"  | 09°19'36.6"   |
| III     | 08.07.18 - 09.07.18                          | Holo reefs N     | 69°06'58.6"  | 14°21'45.7"   |
|         |  | Holo reefs E     | 68°53'22.7"  | 14°46'39.7"   |
|         |  | Holo reefs S     | 68°48'28.9"  | 14°20'16.8"   |
|         |  | Holo reefs W     | 69°00'18.4"  | 13°44'52.3"   |
| IV      | 11.07.18 - 14.07.18                          | Steinavær reef N | 69°16'13.9"  | 16°31'15.4"   |
|         |  | Steinavær reef E | 69°14'59.8"  | 16°50'34.1"   |
|         |  | Steinavær reef S | 69°09'11.1"  | 16°46'25.9"   |
|         |  | Steinavær reef W | 69°12'44.0"  | 16°30'18.5"   |

This is a detailed topographic map of a coastal region in Norway, specifically the Kråkvåg area. The map features a red-shaded triangular area in the upper central part, which is the focus of the study. The map includes contour lines indicating elevation, with labels such as 'Kråkvåg', 'Ijorden', and 'Sørlekka'. There are also various navigational or surveying lines, including a prominent purple line and several green lines. A scale bar at the bottom left indicates a distance of 1000 meters. The map is oriented with North at the top.

**Fig. A3:** Detailed map of Station II reef area 'Nord-Leksa' in the outer Trondheimsfjord in mid-Norway. The red rectangle shows the working area of station II (see coordinates in table A1). Note that the scale is different here (1000 m compared to the previous detailed graph with 10 km scale).



**Fig. A4:** Detailed map of Station III area: Holo reefs in northern Norway further offshore in North Norway. The red rectangle shows the working area of station III (see coordinates in table A1).



**Fig. A5:** Detailed map of Station IV area: the coastal Steinavær Reef, the northernmost Norwegian coral reef station approached during this cruise. The red rectangle shows the working area of station IV (see coordinates in table A1).



High resolution cruise track data can be directly downloaded from:

<https://portal.geomar.de/metadata/leg/show/344884>

#### B. Station list

The complete POS525 ship's logbook (including all sub-stations, e.g. use of rubber boat, etc.) as well as logged data from the board instruments can be downloaded from the DSHIP portal of the Data Management Portal for Kiel Marine Sciences hosted at GEOMAR:

<https://portal.geomar.de/metadata/leg/show/344884>

| Sta-tion | Date     | Time (UTC) | Device       | Action   | Latitude      | Longitude      | Depth (m) |
|----------|----------|------------|--------------|----------|---------------|----------------|-----------|
| 1-1      | 01.07.18 | 6:52       | JAGO test    | in water | 64° 06.608' N | 008° 07.038' E | 299.1     |
| 1-1      | 01.07.18 | 7:13       | JAGO test    | on deck  | 64° 06.783' N | 008° 07.530' E | 299.5     |
| 2-1      | 01.07.18 | 8:10       | CTD + CUBE   | in water | 64° 06.644' N | 008° 07.040' E | 300.2     |
| 2-1      | 01.07.18 | 8:49       | CTD          | on deck  | 64° 06.637' N | 008° 07.085' E | 299.8     |
| 3-1      | 01.07.18 | 8:56       | CTD + CUBE   | in water | 64° 06.643' N | 008° 07.039' E | 299.9     |
| 3-1      | 01.07.18 | 9:53       | CTD          | on deck  | 64° 06.645' N | 008° 07.032' E | 298.7     |
| 4-1      | 01.07.18 | 10:42      | JAGO         | in water | 64° 06.648' N | 008° 07.024' E | 298.7     |
| 4-1      | 01.07.18 | 18:09      | JAGO         | on deck  | 64° 06.858' N | 008° 07.078' E | 299.6     |
| 5-1      | 02.07.18 | 7:03       | JAGO         | in water | 64° 06.615' N | 008° 07.013' E | 300.1     |
| 5-1      | 02.07.18 | 9:30       | JAGO         | on deck  | 64° 06.639' N | 008° 06.986' E | 299.5     |
| 6-1      | 02.07.18 | 10:30      | CTD          | in water | 64° 06.634' N | 008° 06.842' E | 299.5     |
| 6-1      | 02.07.18 | 15:08      | CTD          | on deck  | 64° 06.601' N | 008° 06.987' E | 301.0     |
| 7-1      | 03.07.18 | 6:27       | JAGO         | in water | 64° 06.688' N | 008° 07.033' E | 300.4     |
| 7-1      | 03.07.18 | 9:43       | JAGO + CUBEs | on deck  | 64° 06.781' N | 008° 07.125' E | 301.6     |
| 8-1      | 03.07.18 | 11:04      | CTD          | in water | 64° 06.587' N | 008° 06.953' E | 301.6     |
| 8-1      | 03.07.18 | 11:27      | CTD          | on deck  | 64° 06.572' N | 008° 06.949' E | 300.8     |
| 9-1      | 03.07.18 | 13:30      | JAGO         | in water | 64° 06.577' N | 008° 06.979' E | 297.1     |
| 9-1      | 03.07.18 | 16:31      | JAGO         | on deck  | 64° 06.501' N | 008° 07.241' E | 297.7     |
| 10-1     | 03.07.18 | 16:44      | CTD + pump   | in water | 64° 06.485' N | 008° 07.161' E | 292.3     |
| 10-1     | 03.07.18 | 21:55      | CTD          | on deck  | 64° 06.493' N | 008° 07.259' E | 292.8     |
| 11-1     | 04.07.18 | 7:14       | JAGO         | in water | 63° 36.489' N | 009° 23.002' E | 160.0     |
| 11-1     | 04.07.18 | 7:44       | JAGO         | on deck  | 63° 36.504' N | 009° 22.990' E | 239.3     |
| 12-1     | 04.07.18 | 8:39       | JAGO         | in water | 63° 36.498' N | 009° 22.961' E | 157.8     |
| 12-1     | 04.07.18 | 11:00      | JAGO         | on deck  | 63° 36.401' N | 009° 23.403' E | 157.8     |
| 13-1     | 04.07.18 | 14:14      | CTD          | in water | 63° 36.464' N | 009° 22.782' E | 150.9     |
| 13-1     | 04.07.18 | 14:30      | CTD          | on deck  | 63° 36.422' N | 009° 22.778' E | 190.5     |
| 14-1     | 05.07.18 | 6:02       | CTD          | in water | 63° 36.417' N | 009° 22.864' E | 212.9     |
| 14-1     | 05.07.18 | 6:20       | CTD          | on deck  | 63° 36.406' N | 009° 22.824' E | 212.9     |
| 15-1     | 05.07.18 | 6:34       | JAGO         | in water | 63° 36.420' N | 009° 22.846' E | 208.4     |
| 15-1     | 05.07.18 | 9:21       | JAGO         | on deck  | 63° 36.478' N | 009° 22.835' E | 207.7     |
| 16-1     | 05.07.18 | 10:15      | CTD          | in water | 63° 36.513' N | 009° 22.523' E | 207.7     |
| 16-1     | 05.07.18 | 10:35      | CTD          | on deck  | 63° 36.512' N | 009° 22.566' E | 207.7     |
| 17-1     | 05.07.18 | 13:27      | CTD          | in water | 63° 36.438' N | 009° 22.794' E | 207.7     |
| 17-1     | 05.07.18 | 13:43      | CTD          | on deck  | 63° 36.502' N | 009° 22.718' E | 203.9     |
| 18-1     | 05.07.18 | 14:07      | CTD          | in water | 63° 36.431' N | 009° 22.691' E | 151.5     |
| 18-1     | 05.07.18 | 14:23      | CTD          | on deck  | 63° 36.436' N | 009° 22.706' E | 152.9     |

|      |          |       |            |          |               |                |       |
|------|----------|-------|------------|----------|---------------|----------------|-------|
| 19-1 | 05.07.18 | 14:42 | CTD        | in water | 63° 36.517' N | 009° 22.519' E | 237.6 |
| 19-1 | 05.07.18 | 14:57 | CTD        | on deck  | 63° 36.517' N | 009° 22.477' E | 233.9 |
| 20-1 | 06.07.18 | 6:03  | CTD        | in water | 63° 36.469' N | 009° 22.765' E | 148.8 |
| 20-1 | 06.07.18 | 6:14  | CTD        | on deck  | 63° 36.471' N | 009° 22.787' E | 151.2 |
| 21-1 | 06.07.18 | 6:46  | JAGO       | in water | 63° 36.444' N | 009° 23.298' E | 221.7 |
| 21-1 | 06.07.18 | 9:26  | JAGO       | on deck  | 63° 36.488' N | 009° 22.505' E | 222.0 |
| 22-1 | 06.07.18 | 10:01 | CTD        | in water | 63° 36.517' N | 009° 22.546' E | 222.0 |
| 22-1 | 06.07.18 | 10:17 | CTD        | on deck  | 63° 36.573' N | 009° 22.455' E | 247.7 |
| 23-1 | 08.07.18 | 6:10  | CTD        | in water | 69° 14.923' N | 016° 38.173' E | 246.8 |
| 23-1 | 08.07.18 | 6:35  | CTD        | on deck  | 69° 14.873' N | 016° 38.223' E | 248.9 |
| 24-1 | 08.07.18 | 6:49  | JAGO       | in water | 69° 14.824' N | 016° 38.383' E | 227.6 |
| 24-1 | 08.07.18 | 9:22  | JAGO       | on deck  | 69° 14.915' N | 016° 38.135' E | 231.3 |
| 25-1 | 08.07.18 | 10:13 | CTD        | in water | 69° 14.844' N | 016° 38.407' E | 231.3 |
| 25-1 | 08.07.18 | 10:28 | CTD        | on deck  | 69° 14.892' N | 016° 38.159' E | 249.2 |
| 26-1 | 08.07.18 | 12:28 | CTD        | in water | 69° 14.887' N | 016° 38.467' E | 225.0 |
| 26-1 | 08.07.18 | 12:45 | CTD        | on deck  | 69° 14.843' N | 016° 38.355' E | 233.5 |
| 27-1 | 08.07.18 | 13:09 | JAGO       | in water | 69° 14.811' N | 016° 37.779' E | 283.3 |
| 27-1 | 08.07.18 | 15:53 | JAGO       | on deck  | 69° 14.857' N | 016° 37.979' E | 244.6 |
| 28-1 | 09.07.18 | 6:00  | CTD        | in water | 69° 14.709' N | 016° 38.461' E | 194.2 |
| 28-1 | 09.07.18 | 6:18  | CTD        | on deck  | 69° 14.69° N  | 016° 38.457' E | 201.3 |
| 29-1 | 09.07.18 | 6:38  | JAGO       | in water | 69° 14.683' N | 016° 38.437' E | 202.6 |
| 29-1 | 09.07.18 | 9:17  | JAGO       | on deck  | 69° 14.680' N | 016° 38.164' E | 202.6 |
| 30-1 | 09.07.18 | 10:10 | CTD        | in water | 69° 14.667' N | 016° 38.639' E | 205.3 |
| 30-1 | 09.07.18 | 10:26 | CTD        | on deck  | 69° 14.674' N | 016° 38.558' E | 200.6 |
| 31-1 | 09.07.18 | 11:23 | CTD        | in water | 69° 14.712' N | 016° 38.449' E | 240.0 |
| 31-1 | 09.07.18 | 11:36 | CTD        | on deck  | 69° 14.720' N | 016° 38.489' E | 202.1 |
| 32-1 | 09.07.18 | 12:43 | JAGO       | in water | 69° 14.322' N | 016° 39.082' E | 220.3 |
| 32-1 | 09.07.18 | 15:37 | JAGO       | on deck  | 69° 14.543' N | 016° 38.469' E | 245.0 |
| 33-1 | 09.07.18 | 16:09 | CTD        | in water | 69° 14.69° N  | 016° 38.441' E | 190.4 |
| 33-1 | 09.07.18 | 16:23 | CTD        | on deck  | 69° 14.703' N | 016° 38.474' E | 203.3 |
| 34-1 | 11.07.18 | 7:06  | CTD        | in water | 68° 55.193' N | 014° 24.039' E | 264.3 |
| 34-1 | 11.07.18 | 7:27  | CTD        | on deck  | 68° 55.197' N | 014° 24.167' E | 264.2 |
| 35-1 | 11.07.18 | 8:05  | CTD + pump | in water | 68° 55.091' N | 014° 24.253' E | 267.5 |
| 35-1 | 11.07.18 | 11:44 | CTD        | on deck  | 68° 55.050' N | 014° 24.444' E | 267.5 |
| 36-1 | 11.07.18 | 12:00 | JAGO       | in water | 68° 55.112' N | 014° 24.347' E | 257.0 |
| 36-1 | 11.07.18 | 16:02 | JAGO       | on deck  | 68° 55.097' N | 014° 23.873' E | 265.3 |
| 37-1 | 12.07.18 | 7:26  | CTD        | in water | 68° 55.167' N | 014° 26.186' E | 214.2 |
| 37-1 | 12.07.18 | 7:43  | CTD        | on deck  | 68° 55.156' N | 014° 26.127' E | 216.7 |
| 38-1 | 12.07.18 | 8:15  | CTD        | in water | 68° 54.651' N | 014° 21.909' E | 258.5 |
| 38-1 | 12.07.18 | 8:35  | CTD        | on deck  | 68° 54.617' N | 014° 21.867' E | 260.5 |
| 39-1 | 12.07.18 | 9:03  | CTD        | in water | 68° 55.881' N | 014° 22.693' E | 251.3 |
| 39-1 | 12.07.18 | 9:23  | CTD        | on deck  | 68° 55.835' N | 014° 22.778' E | 251.3 |
| 40-1 | 12.07.18 | 10:38 | CTD + CUBE | in water | 68° 55.058' N | 014° 24.380' E | 267.5 |
| 40-1 | 12.07.18 | 11:33 | CTD        | on deck  | 68° 55.043' N | 014° 24.327' E | 270.0 |
| 41-1 | 12.07.18 | 11:38 | CTD + CUBE | in water | 68° 55.041' N | 014° 24.329' E | 268.9 |
| 41-1 | 12.07.18 | 13:05 | CTD        | on deck  | 68° 55.048' N | 014° 24.463' E | 269.4 |
| 42-1 | 12.07.18 | 13:29 | JAGO       | in water | 68° 55.029' N | 014° 24.440' E | 268.7 |
| 42-1 | 12.07.18 | 17:03 | JAGO       | on deck  | 68° 55.053' N | 014° 23.856' E | 268.6 |
| 43-1 | 13.07.18 | 6:02  | CTD        | in water | 68° 55.093' N | 014° 24.401' E | 264.8 |
| 43-1 | 13.07.18 | 6:21  | CTD        | on deck  | 68° 55.077' N | 014° 24.382' E | 264.8 |

|      |          |       |                |          |               |                |       |
|------|----------|-------|----------------|----------|---------------|----------------|-------|
| 44-1 | 13.07.18 | 6:39  | JAGO           | in water | 68° 54.992' N | 014° 24.525' E | 268.8 |
| 44-1 | 13.07.18 | 11:12 | JAGO           | on deck  | 68° 55.158' N | 014° 24.142' E | 269.3 |
| 45-1 | 13.07.18 | 11:44 | CTD            | on deck  | 68° 54.724' N | 014° 27.095' E | 269.3 |
| 45-1 | 13.07.18 | 12:02 | CTD            | on deck  | 68° 54.733' N | 014° 27.198' E | 223.4 |
| 46-1 | 13.07.18 | 12:41 | CTD            | in water | 68° 55.524' N | 014° 21.128' E | 248.7 |
| 46-1 | 13.07.18 | 13:01 | CTD            | on deck  | 68° 55.517' N | 014° 21.091' E | 247.8 |
| 47-1 | 13.07.18 | 13:37 | JAGO           | in water | 68° 54.873' N | 014° 24.041' E | 270.6 |
| 47-1 | 13.07.18 | 16:13 | JAGO           | on deck  | 68° 54.965' N | 014° 23.920' E | 266.5 |
| 48-1 | 14.07.18 | 6:02  | CTD            | in water | 68° 55.844' N | 014° 22.935' E | 244.3 |
| 48-1 | 14.07.18 | 6:18  | CTD            | on deck  | 68° 55.845' N | 014° 22.934' E | 256.2 |
| 49-1 | 14.07.18 | 6:38  | JAGO           | in water | 68° 55.869' N | 014° 23.055' E | 248.1 |
| 49-1 | 14.07.18 | 9:32  | JAGO           | on deck  | 68° 56.069' N | 014° 23.580' E | 242.8 |
| 50-1 | 14.07.18 | 10:02 | CTD            | in water | 68° 56.311' N | 014° 20.025' E | 241.8 |
| 50-1 | 14.07.18 | 10:17 | CTD            | on deck  | 68° 56.304' N | 014° 19.955' E | 261.5 |
| 51-1 | 14.07.18 | 10:36 | CTD            | in water | 68° 56.307' N | 014° 21.565' E | 261.5 |
| 51-1 | 14.07.18 | 10:54 | CTD            | on deck  | 68° 56.327' N | 014° 21.523' E | 227.2 |
| 52-1 | 14.07.18 | 11:16 | CTD            | in water | 68° 56.374' N | 014° 24.249' E | 229.0 |
| 52-1 | 14.07.18 | 11:34 | CTD            | on deck  | 68° 56.340' N | 014° 24.295' E | 266.8 |
| 53-1 | 14.07.18 | 12:34 | JAGO           | in water | 68° 55.048' N | 014° 24.461' E | 286.8 |
| 53-1 | 14.07.18 | 16:06 | JAGO + CUBEs   | on deck  | 68° 55.528' N | 014° 24.724' E | 298.4 |
| 53-2 | 16.07.18 | 7:41  | JAGO           | in water | 64° 06.609' N | 008° 06.936' E | 298.4 |
| 53-2 | 16.07.18 | 11:06 | JAGO           | on deck  | 64° 06.696' N | 008° 07.075' E | 300.0 |
| 54-1 | 16.07.18 | 11:19 | CTD            | in water | 64° 06.668' N | 008° 07.203' E | 300.8 |
| 54-1 | 16.07.18 | 11:43 | CTD            | on deck  | 64° 06.613' N | 008° 07.080' E | 300.0 |
| 55-1 | 16.07.18 | 12:28 | CTD + CUBE     | in water | 64° 06.628' N | 008° 07.181' E | 301.0 |
| 55-1 | 16.07.18 | 13:43 | CTD            | on deck  | 64° 06.627' N | 008° 07.174' E | 301.0 |
| 56-1 | 16.07.18 | 13:48 | CTD + CUBE     | in water | 64° 06.626' N | 008° 07.186' E | 301.0 |
| 56-1 | 16.07.18 | 14:49 | CTD            | on deck  | 64° 06.633' N | 008° 07.169' E | 301.0 |
| 56-2 | 16.07.18 | 15:07 | JAGO           | in water | 64° 06.657' N | 008° 07.059' E | 301.0 |
| 56-2 | 16.07.18 | 18:45 | JAGO           | on deck  | 64° 06.633' N | 008° 07.033' E | 293.4 |
| 57-1 | 17.07.18 | 6:00  | CTD            | in water | 64° 06.505' N | 008° 07.015' E | 287.0 |
| 57-1 | 17.07.18 | 6:22  | CTD            | on deck  | 64° 06.508' N | 008° 07.049' E | 295.0 |
| 58-1 | 17.07.18 | 6:37  | JAGO           | in water | 64° 06.558' N | 008° 06.906' E | 283.7 |
| 58-1 | 17.07.18 | 9:08  | JAGO           | on deck  | 64° 06.581' N | 008° 07.083' E | 347.8 |
| 59-1 | 17.07.18 | 10:17 | CTD            | in water | 64° 06.373' N | 008° 07.914' E | 347.2 |
| 59-1 | 17.07.18 | 10:42 | CTD            | on deck  | 64° 06.367' N | 008° 07.908' E | 345.2 |
| 60-1 | 17.07.18 | 10:58 | CTD            | in water | 64° 06.295' N | 008° 07.316' E | 344.0 |
| 60-1 | 17.07.18 | 11:15 | CTD            | on deck  | 64° 06.305' N | 008° 07.363' E | 343.9 |
| 61-1 | 17.07.18 | 11:38 | CTD            | in water | 64° 06.149' N | 008° 07.556' E | 341.3 |
| 61-1 | 17.07.18 | 11:56 | CTD            | on deck  | 64° 06.164' N | 008° 07.472' E | 302.5 |
| 62-1 | 17.07.18 | 12:30 | JAGO           | in water | 64° 06.902' N | 008° 07.733' E | 297.8 |
| 62-1 | 17.07.18 | 15:07 | JAGO           | on deck  | 64° 06.665' N | 008° 07.556' E | 298.7 |
| 62-2 | 17.07.18 | 15:18 | CTD + pump     | in water | 64° 06.662' N | 008° 07.541' E | 288.1 |
| 62-2 | 17.07.18 | 21:51 | CTD            | on deck  | 64° 06.777' N | 008° 08.568' E | 298.1 |
| 63-1 | 18.07.18 | 7:43  | JAGO + baskets | in water | 64° 06.655' N | 008° 07.181' E | 299.1 |
| 63-1 | 18.07.18 | 14:10 | JAGO + CUBEs   | on deck  | 64° 06.640' N | 008° 07.827' E | 299.1 |

## C. Sample list

| Date     | Site       | Dive # | Sample #      | Species           | Depth (m) | Latitude  | Longitude | Sub-sample type     |
|----------|------------|--------|---------------|-------------------|-----------|-----------|-----------|---------------------|
| 01.07.18 | Sula Reef  | J01    | POS525-J01-1  | <i>Lophelia</i>   | 300       | 64°04.64  | 08°06.99  | R, G                |
| 02.07.18 | Sula Reef  | J02    | POS525-J02-1  | <i>Lophelia</i>   | 289       | 64°06.603 | 8°07.206  | R, G                |
| 02.07.18 | Sula Reef  | J02    | POS525-J02-2  | <i>Lophelia</i>   | 289       | 64°06.603 | 8°07.206  | R, G                |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-1  | <i>Lophelia</i>   | 280       | 64°05.557 | 08°07.208 | R, G, FA, IR, SM    |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-2  | <i>Lophelia</i>   | 280       | 64°05.570 | 08°07.297 | R, G, FA, IR, SM    |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-3  | <i>Lophelia</i>   | 280       | 64°05.548 | 08°07.226 | R, G, FA, IR, SM    |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-4  | <i>Lophelia</i>   | 280       | 64°05.538 | 08°07.233 | R, G, FA, IR, SM    |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-5  | <i>Lophelia</i>   | 278       | 64°05.530 | 08°07.235 | R, G, FA, IR, SM    |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-6  | <i>Lophelia</i>   | 281       | 64°05.530 | 08°07.235 | R, G, FA            |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-7  | <i>Madrepora</i>  | 281       | 64°05.530 | 08°07.235 | R, G, FA            |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-8  | <i>Lophelia</i>   | 278       | 64°05.531 | 08°07.243 | R, G, FA            |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-9  | <i>Lophelia</i>   | 278       | 64°05.531 | 08°07.243 | R, G, FA            |
| 03.07.18 | Sula Reef  | J04    | POS525-J04-10 | <i>Mycale sp.</i> | 273       | 64°05.553 | 08°07.204 | Biomedicine         |
| 05.07.18 | Nordleksa  | J07    | POS525-J07-1  | <i>Lophelia</i>   | 278       | 63°36.455 | 09°22.786 | R, G                |
| 05.07.18 | Nordleksa  | J07    | POS525-J07-1  | Water             | 155       | 63°36.455 | 09°22.786 | TA, DIC, Nut, Isot. |
| 05.07.18 | Nordleksa  | J07    | POS525-J07    | <i>Acesta</i>     | 155       | 63°36.454 | 09°22.771 | Stable Isotopes     |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-1  | <i>Lophelia</i>   | 166       | 63°36.46  | 09°22.86  | G, FA, SM           |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-2  | <i>Lophelia</i>   | 165       | 63°36.46  | 09°22.86  | R, G, FA, SM        |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-3  | <i>Lophelia</i>   | 163       | 63°36.46  | 09°22.86  | R, G, FA, SM        |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-4  | <i>Lophelia</i>   | 163       | 63°36.46  | 09°22.86  | R, G, FA            |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-5  | <i>Lophelia</i>   | 163       | 63°36.46  | 09°22.86  | R, G, FA, SM        |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-6  | <i>Lophelia</i>   | 164       | 63°36.46  | 09°22.86  | R, G, FA, SM        |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-7  | <i>Lophelia</i>   | 163       | 63°36.46  | 09°22.86  | R, G, FA            |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-8  | <i>Lophelia</i>   | 165       | 63°36.470 | 09°22.829 | R, G, FA            |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-9  | <i>Lophelia</i>   | 165       | 63°36.470 | 09°22.829 | R, G, FA            |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-10 | <i>Lophelia</i>   | 166       | 63°36.470 | 09°22.829 | Biomedicine         |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-11 | <i>Lophelia</i>   | 163       | 63°36.470 | 09°22.829 | Biomedicine         |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-12 | <i>Lophelia</i>   | 163       | 63°36.470 | 09°22.829 | Biomedicine         |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-13 | <i>Lophelia</i>   | 163       | 63°36.470 | 09°22.829 | Biomedicine         |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-14 | <i>Mycale</i>     | 164       | 63°36.470 | 09°22.829 | Biomedicine         |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-15 | Ascidian          | 166       | 63°36.470 | 09°22.829 | Biomedicine         |
| 06.07.18 | Nordleksa  | J08    | POS525-J08-16 | red sponge        | 152       | 63°36.470 | 09°22.829 | Biomedicine         |
| 08.07.18 | Steinavaer | J09    | POS525-J09-1  | Water             | 245       | 69°14.903 | 16°38.274 | TA, Nut, DIC, Isot. |
| 08.07.18 | Steinavaer | J09    | POS525-J09-1  | <i>Lophelia</i>   | 245       | 69 14.903 | 16 38.274 | R, G, FA            |
| 08.07.18 | Steinavaer | J09    | POS525-J09-2a | <i>Lophelia</i>   | 245       | 69 14.903 | 16 38.274 | R, G, FA            |
| 08.07.18 | Steinavaer | J09    | POS525-J09-2b | <i>Madrepora</i>  | 245       | 69 14.903 | 16 38.274 | R, G, FA            |
| 08.07.18 | Steinavaer | J09    | POS525-J09-3  | <i>Lophelia</i>   | 245       | 69 14.903 | 16 38.274 | R, G, FA            |
| 08.07.18 | Steinavaer | J09    | POS525-J09-4  | Sponge            | 245       | 69°14.895 | 16°38.337 | Biomedicine         |
| 08.07.18 | Steinavaer | J09    | POS525-J09-5  | Ascidian          | 244       | 69°14.895 | 16°38.337 | Biomedicine         |
| 08.07.18 | Steinavaer | J10    | POS525-J10-1  | Water             | 245       | 69°14.910 | 16°38.227 | TA, DIC, Nut, Isot. |
| 08.07.18 | Steinavaer | J10    | POS525-J10-1  | <i>Lophelia</i>   | 244       | 69°14.910 | 16°38.257 | R, G, FA            |



|          |            |     |              |                  |     |           |           |                     |
|----------|------------|-----|--------------|------------------|-----|-----------|-----------|---------------------|
| 08.07.18 | Steinavaer | J10 | POS525-J10-2 | <i>Lophelia</i>  | 244 | 69°14.910 | 16°38.257 | R, G, FA            |
| 08.07.18 | Steinavaer | J10 | POS525-J10-3 | <i>Lophelia</i>  | 243 | 69°14.886 | 16°38.286 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-1 | Water            | 200 | 69°14.676 | 16°38.466 | TA, DIC, Nut, Isot. |
| 09.07.18 | Steinavaer | J11 | POS525-J11-1 | <i>Lophelia</i>  | 205 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-2 | <i>Lophelia</i>  | 205 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-3 | <i>Lophelia</i>  | 200 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-4 | <i>Lophelia</i>  | 200 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-5 | <i>Lophelia</i>  | 195 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-6 | <i>Lophelia</i>  | 219 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-7 | <i>Lophelia</i>  | 205 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-8 | Sponge           | 205 | 69°14.679 | 16°38.465 | Biomedicine         |
| 09.07.18 | Steinavaer | J11 | POS525-J11-8 | <i>Lophelia</i>  | 205 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J11 | POS525-J11-9 | <i>Lophelia</i>  | 205 | 69°14.679 | 16°38.465 | R, G, FA            |
| 09.07.18 | Steinavaer | J12 | POS525-J12-1 | <i>Lophelia</i>  | 237 | 69°14.348 | 16°38.576 | R, G, FA            |
| 09.07.18 | Steinavaer | J12 | POS525-J12-2 | <i>Lophelia</i>  | 237 | 69°14.348 | 16°38.576 | R, G, FA            |
| 09.07.18 | Steinavaer | J12 | POS525-J12-3 | <i>Lophelia</i>  | 237 | 69°14.348 | 16°38.576 | R, G, FA            |
| 09.07.18 | Steinavaer | J12 | POS525-J12-4 | <i>Lophelia</i>  | 237 | 69°14.348 | 16°38.576 | R, G, FA            |
| 11.07.18 | Hola       | J13 | POS525-J13-1 | <i>Lophelia</i>  | 260 | 68°55.097 | 14°24.139 | R, G, FA            |
| 11.07.18 | Hola       | J13 | POS525-J13-2 | <i>Lophelia</i>  | 260 | 68°55.097 | 14°24.139 | R, G, FA            |
| 11.07.18 | Hola       | J13 | POS525-J13-3 | <i>Lophelia</i>  | 260 | 68°55.097 | 14°24.139 | R, G, FA            |
| 11.07.18 | Hola       | J13 | POS525-J13-4 | <i>Lophelia</i>  | 260 | 68°55.097 | 14°24.139 | R, G, FA            |
| 11.07.18 | Hola       | J13 | POS525-J13-5 | <i>Lophelia</i>  | 260 | 68°55.097 | 14°24.139 | R, G, FA            |
| 11.07.18 | Hola       | J13 | POS525-J13-6 | <i>Lophelia</i>  | 260 | 68°55.097 | 14°24.139 | R, G, FA            |
| 13.07.18 | Hola       | J16 | POS525-J16-1 | Water            | 258 | 68°54.930 | 14°24.115 | TA, DIC, Nut, Isot. |
| 13.07.18 | Hola       | J16 | POS525-J16-1 | <i>Lophelia</i>  | 257 | 68°54.930 | 14°24.115 | R, G, FA            |
| 13.07.18 | Hola       | J16 | POS525-J16-2 | <i>Lophelia</i>  | 258 | 68°54.930 | 14°24.115 | R, G, FA            |
| 13.07.18 | Hola       | J16 | POS525-J16-3 | <i>Lophelia</i>  | 257 | 68°54.930 | 14°24.115 | R, G, FA            |
| 13.07.18 | Hola       | J16 | POS525-J16-4 | <i>Lophelia</i>  | 257 | 68°54.930 | 14°24.115 | R, G, FA            |
| 13.07.18 | Hola       | J16 | POS525-J16-5 | <i>Lophelia</i>  | 257 | 68°54.930 | 14°24.115 | R, G, FA            |
| 13.07.18 | Hola       | J16 | POS525-J16-6 | <i>Lophelia</i>  | 258 | 68°54.930 | 14°24.115 | R, G, FA            |
| 13.07.18 | Hola       | J16 | POS525-J16-7 | <i>Lophelia</i>  | 258 | 68°54.930 | 14°24.115 | R, G, FA            |
| 13.07.18 | Hola       | J16 | POS525-J16-8 | Sponge           | 258 | 68°54.930 | 14°24.115 | Biomedicine         |
| 14.07.18 | Hola       | J17 | POS525-J17-1 | Water            | 250 | 68°56.073 | 14°13.405 | TA, DIC, Nut, Isot. |
| 14.07.18 | Hola       | J17 | POS525-J17-1 | <i>Lophelia</i>  | 250 | 68°56.073 | 14°13.405 | R, G, FA            |
| 14.07.18 | Hola       | J17 | POS525-J17-2 | <i>Lophelia</i>  | 250 | 68°56.073 | 14°13.405 | R, G, FA            |
| 14.07.18 | Hola       | J17 | POS525-J17-3 | <i>Lophelia</i>  | 251 | 68°56.057 | 14°13.411 | R, G, FA            |
| 14.07.18 | Hola       | J17 | POS525-J17-4 | Sponge           | 251 | 68°56.062 | 14°13.413 | Biomedicine         |
| 14.07.18 | Hola       | J17 | POS525-J17-5 | Sponge           | 251 | 68°56.062 | 14°13.413 | Biomedicine         |
| 16.07.18 | Sula Reef  | J19 | POS525-J19-1 | <i>Lophelia</i>  | 302 | 64°06.592 | 08°07.027 | R, G, FA            |
| 16.07.18 | Sula Reef  | J19 | POS525-J19-2 | <i>Lophelia</i>  | 303 | 64°06.593 | 08°06.995 | R, G, FA            |
| 16.07.18 | Sula Reef  | J19 | POS525-J19-3 | <i>Madrepora</i> | 302 | 64°06.593 | 08°06.995 | R, G, FA            |
| 16.07.18 | Sula Reef  | J19 | POS525-J19-4 | <i>Lophelia</i>  | 301 | 64°06.593 | 08°06.995 | R, G, FA            |
| 16.07.18 | Sula Reef  | J19 | POS525-J19-5 | <i>Lophelia</i>  | 301 | 64°06.593 | 08°06.995 | R, G, FA            |
| 16.07.18 | Sula Reef  | J19 | POS525-J19-6 | <i>Madrepora</i> | 302 | 64°06.592 | 08°07.037 | R, G, FA            |
| 16.07.18 | Sula Reef  | J19 | POS525-J19-7 | <i>Madrepora</i> | 302 | 64°06.593 | 08°06.995 | R, G, FA            |

|          |           |     |              |                  |     |           |           |              |
|----------|-----------|-----|--------------|------------------|-----|-----------|-----------|--------------|
| 16.07.18 | Sula Reef | J19 | POS525-J19-8 | <i>Madrepora</i> | 303 | 64°06.593 | 08°06.995 | R, G, FA     |
| 18.07.18 | Sula Reef | J23 | POS525-J23-1 | Water            | 290 | 64°06.601 | 08°07.108 | TA, DIC, Nut |

Sample list of all JAGO samples collected at the four different reef sites with JAGO dive number, coordinates and depth of the collection location, the specific sample reference (sample #) and the specific purpose (i.e. 'sub-sample type') of each sample (abbreviations: *R*: reproduction, *G*: genetics, *FA*: fatty acids (and lipids), *IR*: incubation / respiration samples for on-board measurements, *SM*: samples for stress marker analyses, Biomedicine: samples for biomedical purposes, and samples for seawater parameter analyses such as *TA*: total alkalinity, *DIC*: dissolved inorganic carbon, *Nut*: nutrients, *Isot.*: samples for several stable isotope and trace element analyses).